plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the number of them which are more intense than the spot from the standard solution (2) is not more than two. Spray evenly acidic potassium permanganate TS on the plate: any spot other than the principal spot does not appear.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

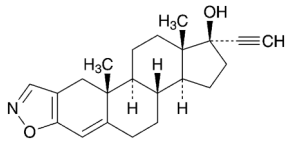
Assay Weigh accurately about 0.2 g of Cytarabine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 12.16 mg of $C_9H_{13}N_3O_5$

Containers and storage Containers—Tight containers.

Danazol

ダナゾール



$C_{22}H_{27}NO_2$: 337.46
17α-Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol
[*17230-88-5*]

Danazol, when dried, contains not less than 98.5% and not more than 101.0% of danazol ($C_{22}H_{27}NO_2$).

Description Danazol occurs as a white to pale yellow crystalline powder.

It is soluble in acetone, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 225°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Danazol in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Danazol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Danazol as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Danazol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +8 – +11° (after drying, 0.25 g, ethanol (99.5), 50 mL, 100 mm).

Purity **(1)** Chloride ⟨*1.03*⟩—To 2.0 g of Danazol add 80 mL of water, shake well, and boil for 5 minutes. After cooling, add water to make 100 mL, and filter through a glass filter (G4). Discard the first 30 mL of the filtrate, take 40 mL of the subsequent filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Danazol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Danazol in 4 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetone to make exactly 200 mL. Pipet 4 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.2% (1 g, in vacuum, phosphorous (V) oxide, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Danazol and Danazol RS, previously dried, dissolve separately in ethanol (95) to make exactly 50 mL. Pipet 2 mL each of these solutions, add ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 285 nm.

Amount (mg) of danazol ($C_{22}H_{27}NO_2$)
= $M_S \times A_T/A_S$

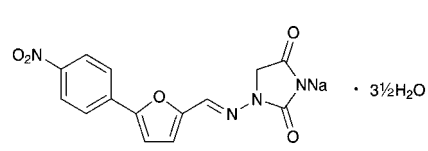
M_S: Amount (mg) of Danazol RS taken

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dantrolene Sodium Hydrate

ダントロレンナトリウム水和物



$C_{14}H_9N_4NaO_5.3\frac{1}{2}H_2O$: 399.29
Monosodium 3-[5-(4-nitrophenyl)furan-2-ylmethylene]amino-2,5-dioxo-1,3-imidazolidinate hemiheptahydrate
[*14663-23-1*, anhydride]

Dantrolene Sodium Hydrate contains not less than 98.0% of dantrolene sodium ($C_{14}H_9N_4NaO_5$: 336.23), calculated on the anhydrous basis.

Description Dantrolene Sodium Hydrate occurs as a yellowish orange to deep orange, crystalline powder.

It is soluble in propylene glycol, sparingly soluble in methanol, slightly soluble in ethanol (95), very slightly soluble in water and in acetic acid (100), and practically insoluble in acetone, in tetrahydrofuran and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Dantrolene Sodium Hydrate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dantrolene Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Dantrolene Sodium Hydrate add 20 mL of water and 2 drops of acetic acid (100), shake well, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Alkalinity—To 0.7 g of Dantrolene Sodium Hydrate add 10 mL of water, shake well, and centrifuge or filter through a membrane filter. To 5 mL of the supernatant liquid or the filtrate add 45 mL of water, 3 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS: a red color is not produced.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Dantrolene Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related Substances—Dissolve 50 mg of Dantrolene Sodium Hydrate in 20 mL of tetrahydrofuran and 2 mL of acetic acid (100), add ethanol (99.5) to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from these solutions by the automatic integration method: the total area of peaks other than dantrolene obtained from the sample solution is not larger than the peak area of dantrolene obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of hexane, acetic acid (100) and ethanol (99.5) (90:10:9).

Flow rate: Adjust so that the retention time of dantrolene is about 8 minutes.

Selection of column: Dissolve 5 mg of Dantrolene Sodium Hydrate and 0.1 g of theophylline in 20 mL of tetrahydrofuran and 2 mL of acetic acid (100), and add ethanol (99.5) to make 100 mL. To 10 mL of this solution add ethanol (99.5) to make 100 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of theophylline and dantrolene in this order with the resolution between these peaks being not less than 6.

Detection sensitivity: Adjust so that the peak height of dantrolene from 10 μL of the standard solution is 10 to 40% of the full scale.

Time span of measurement: About twice as long as the retention time of dantrolene, beginning after the solvent peak.

Water <2.48> 14.5 – 17.0% (0.2 g, volumetric titrotion, direct titration).

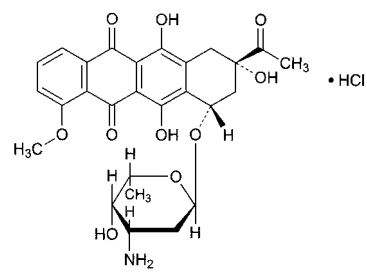
Assay Weigh accurately about 0.7 g of Dantrolene Sodium Hydrate, dissolve in 180 mL of a mixture of propylene glycol and acetone (1:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.62 mg of $C_{14}H_9N_4NaO_5$

Containers and storage Containers—Tight containers.

Daunorubicin Hydrochloride

ダウノルビシン塩酸塩



$C_{27}H_{29}NO_{10}$.HCl: 563.98

(2*S*,4*S*)-2-Acetyl-4-(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyloxy)-2,5,12-trihydroxy-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride

[*23541-50-6*]

Daunorubicin Hydrochloride is the hydrochloride of an anthracycline substance having antitumor activity produced by the growth of *Streptomyces peucetius* or *Streptomyces coeruleorubidus*.

It contains not less than 940 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the dried basis. The potency of Daunorubicin Hydrochloride is expressed as mass (potency) of daunorubicin hydrochloride ($C_{27}H_{29}NO_{10}$.HCl).

Description Daunorubicin Hydrochloride occurs as a red powder.

It is soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Daunorubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Daunorubicin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Daunorubicin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Daunorubicin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the

same wave numbers.

(3) A solution of Daunorubicin Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +250 – +275° (15 mg calculated on the dried basis, methanol, 10 mL, 100 mm).

pH <*2.54*> Dissolve 0.15 g of Daunorubicin Hydrochloride in 30 mL of water: the pH of the solution is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 20 mg of Daunorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Daunorubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 50 mg of Daunorubicin Hydrochloride, dissolve in diluted acetonitrile (43 in 100) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Daunorubicin Hydrochloride RS, and dissolve in diluted acetonitrile (43 in 100) to make exactly 50 mL. Pipet 1 mL of this solution, add diluted acetonitrile (43 in 100) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, weigh accurately about 5 mg of Doxorubicin Hydrochloride RS, and dissolve in diluted acetonitrile (43 in 100) to make exactly 100 mL. Pipet 1 mL of this solution, add diluted acetonitrile (43 in 100) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 5 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of related substances by the following equations: each amount of each peak, having a relative retention time of about 0.3, about 0.6, about 0.7, about 0.8, about 1.7 and about 2.0 to daunorubicin, is not more than 1.3%, not more than 1.0%, not more than 0.3%, not more than 0.5%, not more than 0.4%and not more than 0.5%, respectively, and the amount of doxorubicin is not more than 0.4%. Furthermore, the total amount of the peaks, other than daunorubicin and the peaks mentioned above, is not more than 0.4%. For the area of the peak, having a relative retention time of about 0.3 to daunorubicin, multiply the relative response factor, 0.7.

Each amount (%) of related substances other than doxorubicin
$= M_{S1}/M_T \times A_T/A_{S1} \times 1/2$

M_{S1}: Amount (mg) of Daunorubicin Hydrochloride RS taken
M_T: Amount (mg) of Daunorubicin Hydrochloride taken
A_{S1}: Peak area of daunorubicin obtained from the standard solution (1)
A_T: Peak area of each related substance obtained from the sample solution

Amount (%) of doxorubicin $= M_{S2}/M_T \times A_T/A_{S2} \times 5$

M_{S2}: Amount (mg) of Doxorubicin Hydrochloride RS taken
M_T: Amount (mg) of Daunorubicin Hydrochloride taken
A_{S2}: Peak area of doxorubicin obtained from the standard solution (2)
A_T: Peak area of doxorubicin obtained from the sample solution

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate and 2.25 g of phosphoric acid in water to make 1000 mL. To 570 mL of this solution add 430 mL of acetonitrile.
Flow rate: Adjust so that the retention time of daunorubicin is about 26 minutes.
Time span of measurement: About 2 times as long as the retention time of daunorubicin.
System suitability—
Test for required detectability: To exactly 1 mL of the standard solution (1) add diluted acetonitrile (43 in 100) to make exactly 10 mL. Confirm that the peak area of daunorubicin obtained with 5 μL of this solution is equivalent to 7 to 13% of that obtained with 5 μL of the standard solution (1).
System performance: Dissolve 5 mg each of Daunorubicin Hydrochloride and doxorubicin hydrochloride in 25 mL of diluted acetonitrile (43 in 100). To 1 mL of this solution add diluted acetonitrile (43 in 100) to make 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, doxorubicin and daunorubicin are eluted in this order with the resolution between these peaks being not less than 13.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of daunorubicin is not more than 3.0%.

Loss on drying <*2.41*> Not more than 7.5% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Daunorubicin Hydrochloride and Daunorubicin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of daunorubicin to that of the internal standard.

Amount [μg (potency)] of daunorubicin hydrochloride ($C_{27}H_{29}NO_{10}$.HCl)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Daunorubicin Hydrochloride RS taken

Internal standard solution—A solution of 2-naphthalenesulfonic acid in the mobile phase (1 in 100).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Adjust the pH of a mixture of water and

acetonitrile (31:19) to 2.2 with phosphoric acid.

Flow rate: Adjust so that the retention time of daunorubicin is about 9 minutes.

System suitability—

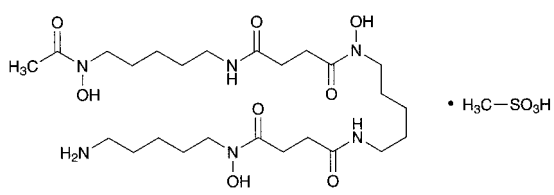
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and daunorubicin are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of daunorubicin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Deferoxamine Mesilate

デフェロキサミンメシル酸塩



$C_{25}H_{48}N_6O_8.CH_4O_3S$: 656.79

N-[5-(Acetylhydroxyamino)pentyl]-*N'*-(5-{3-[(5-aminopentyl)hydroxycarbamoyl]propanoylamino}pentyl)-*N'*-hydroxysuccinamide monomethanesulfonate

[*138-14-7*]

Deferoxamine Mesilate contains not less than 98.0% and not more than 102.0% of deferoxamine mesilate ($C_{25}H_{48}N_6O_8.CH_4O_3S$), calculated on the anhydrous basis.

Description Deferoxamine Mesilate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5), in 2-propanol and in diethyl ether.

Melting point: about 147°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Deferoxamine Mesilate (1 in 500) add 1 drop of iron (III) chloride TS: a deep red color develops.

(2) A 50 mg portion of Deferoxamine Mesilate responds to the Qualitative Tests <*1.09*> (1) for mesilate.

(3) Determine the infrared absorption spectrum of Deferoxamine Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Deferoxamine Mesilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the pH of this solution is between 3.5 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <*1.03*>—Perform the test with 1.0 g of Deferoxamine Mesilate. Prepare the control solution with 0.90 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.032%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of Deferoxamine Mesilate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.040%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Deferoxamine Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Deferoxamine Mesilate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(6) Related substances—Dissolve 50 mg of Deferoxamine Mesilata in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of peaks other than deferoxamine obtained from the sample solution is not larger than the peak area of deferoxamine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.32 g of diammonium hydrogen phosphate, 0.37 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.08 g of sodium 1-heptanesulfonate in 950 mL of water, and adjust the pH of this solution to 2.8 with phosphoric acid. To 800 mL of this solution add 100 mL of 2-propanol.

Flow rate: Adjust so that the retention time of deferoxamine is about 15 minutes.

Time span of measurement: About two times as long as the retention time of deferoxamine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of deferoxamine obtained from 20 μL of this solution is equivalent to 1.5 to 2.5% of that of deferoxamine obtained from 20 μL of the standard solution.

System performance: Dissolve 16 mg of Deferoxamine Mesilate and 4 mg of methyl parahydroxybenzoate in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, deferoxamine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of deferoxamine is not more than 3.0%.

Water <*2.48*> Not more than 2.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg of Deferoxamine Mesilate and Deferoxamine Mesilate RS (previously deter-

mine the water ⟨*2.48*⟩ in the same manner as Deferoxamine Mesilate), dissolve each in 20 mL of water, add exactly 10 mL of 0.05 mol/L sulfuric acid TS, and add water to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of 0.05 mol/L sulfuric acid TS and exactly 0.2 mL of iron (III) chloride TS, then add water to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution prepared by adding 0.05 mol/L sulfuric acid TS to 0.2 mL of iron (III) chloride TS to make exactly 50 mL as the blank, and determine the absorbances, A_T and A_S, of each solution from the sample solution and the standard solution at 430 nm.

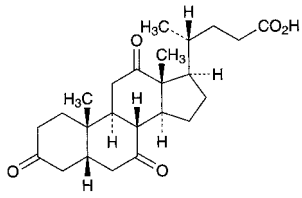
Amount (mg) of deferoxamine mesilate ($C_{25}H_{48}N_6O_8.CH_4O_3S$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Deferoxamine Mesilate RS taken, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Dehydrocholic Acid

デヒドロコール酸



$C_{24}H_{34}O_5$: 402.52
3,7,12-Trioxo-5β-cholan-24-oic acid
[*81-23-2*]

Dehydrocholic Acid, when dried, contains not less than 98.5% of dehydrocholic acid ($C_{24}H_{34}O_5$).

Description Dehydrocholic Acid occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 5 mg of Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and a blue-green fluorescence.

(2) To 0.02 g of Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +29 – +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 233 – 242°C

Purity **(1)** Odor—To 2.0 g of Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.

(2) Clarity and color of solution—To 0.10 g of Dehydrocholic Acid, previously powdered in a mortar, add 30 mL of ethanol (95), and dissolve by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride ⟨*1.03*⟩—To 2.0 g of Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate ⟨*1.14*⟩—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Dehydrocholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes. Cool, filter, and wash with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

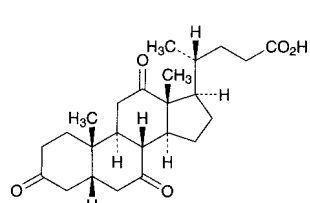
Assay Weigh accurately about 0.5 g of Dehydrocholic Acid, previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS $= 40.25$ mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Well-closed containers.

Purified Dehydrocholic Acid

精製デヒドロコール酸



$C_{24}H_{34}O_5$: 402.52
3,7,12-Trioxo-5β-cholan-24-oic acid
[*81-23-2*]

Purified Dehydrocholic Acid, when dried, contains not less than 99.0% of dehydrocholic acid ($C_{24}H_{34}O_5$).

Description Purified Dehydrocholic Acid occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 5 mg of Purified Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and blue-green fluorescence.

(2) To 0.02 g of Purified Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +29 – +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 237 – 242°C

Purity **(1)** Odor—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.

(2) Clarity and color of solution—Dissolve 0.10 g of Purified Dehydrocholic Acid, previously powdered in a mortar, in 30 mL of ethanol (95) by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride ⟨*1.03*⟩—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate ⟨*1.14*⟩—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Purified Dehydrocholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes, cool, filter, and wash the filter with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Purified Dehydrocholic Acid , previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, then titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Well-closed containers.

Dehydrocholic Acid Injection

Dehydrocholate Sodium Injection

デヒドロコール酸注射液

Dehydrocholic Acid Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of dehydrocholic acid ($C_{24}H_{34}O_5$: 402.52).

Method of preparation Dissolve Purified Dehydrocholic Acid in a solution of Sodium Hydroxide, and prepare as directed under Injections.

Description Dehydrocholic Acid Injection is a clear, colorless to light yellow liquid, and has a bitter taste.

pH: 9 – 11

Identification Transfer a volume of Dehydrocholic Acid Injection, equivalent to 0.1 g of Purified Dehydrocholic Acid, to a separator, and add 10 mL of water and 1 mL of dilute hydrochloric acid: a white precipitate is produced. Extract the mixture with three 15-mL portions of chloroform, combine all the chloroform extracts, evaporate the chloroform on a water bath, and dry the residue at 105°C for 1 hour: the residue so obtained melts ⟨*2.60*⟩ between 235°C and 242°C.

Purity Heavy metals ⟨*1.07*⟩—Evaporate a volume of Dehydrocholic Acid Injection, equivalent to 1.0 g of Purified Dehydrocholic Acid, on a water bath to dryness. Proceed with the residue according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.30 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer an exactly measured volume of Dehydrocholic Acid Injection, equivalent to about 0.5 g of dehydrocholic acid ($C_{24}H_{34}O_5$), to a 100-mL separator, and add, if necessary, water to make 25 mL. Add 2 mL of hydrochloric acid, and extract with 25-mL, 20-mL and 15-mL portions of chloroform successively. Combine the chloroform extracts, wash with cold water until the washings become negative to acid, and evaporate the chloroform on a water bath. Dissolve the residue in 40 mL of neutralized ethanol and 20 mL of water by warming. Add 2 drops of phenolphthalein TS to this solution, titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

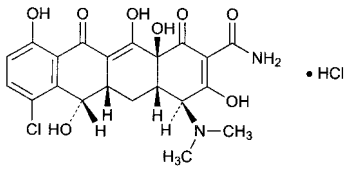
Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Demethylchlortetracycline Hydrochloride

デメチルクロルテトラサイクリン塩酸塩



$C_{21}H_{21}ClN_2O_8$.HCl: 501.31
(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-Chloro-4-dimethylamino-3,6,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride
[*64-73-3*]

Demethylchlortetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of the mutant of *Streptomyces aureofaciens*.

It contains not less than 900 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Demethylchlortetracycline Hydrochloride is expressed as mass (potency) of demethylchlortetracycline hydrochloride ($C_{21}H_{21}ClN_2O_8$.HCl).

Description Demethylchlortetracycline Hydrochloride occurs as a yellow crystalline powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

Identification **(1)** Dissolve 40 mg of Demethylchlortetracycline Hydrochloride in 250 mL of water. To 10 mL of this solution add 85 mL of water and 5 mL of a solution of sodium hydroxide (1 in 5). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Demethylchlortetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Demethylchlortetracycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Demethylchlortetracycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Demethylchlortetracycline Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -248 – $-263°$ (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Demethylchlortetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 2.0 and 3.0.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Demethylchlortetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Demethylchlortetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 25 mg of Demethylchlortetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area obtained from the chromatograms of these solutions by the automatic integration method: each peak area other than demethylchlortetracycline obtained from the sample solution is not larger than 1.2 times that of demethylchlortetracycline obtained from the standard solution, and the sum of the areas of the peaks other than demethylchlortetracycline is not larger than 2 times the peak area of demethylchlortetracycline from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of demethylchlortetracycline, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure 10 mL of the standard solution, add 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of demethylchlortetracycline obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

Loss on drying <*2.41*> Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Demethylchlortetracycline Hydrochloride and Demethylchlortetracycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve each in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of demethylchlortetracycline in each solution.

Amount [μg (potency)] of demethylchlortetracycline hydrochloride ($C_{21}H_{21}ClN_2O_8$.HCl)
$= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Demethylchlortetracycline Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate, 1.5 g of tetrabutylammonium hydrogensulfate and 0.4 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 300 mL of water, and adjust the pH to 8.5 with sodium hydroxide TS. To this solution add 75.0 g of *t*-butanol and water to make 1000 mL.

Flow rate: Adjust so that the retention time of demethylchlortetracycline is about 8 minutes.

System suitability—

System performance: Heat 10 mL of the standard solution on a water bath for 60 minutes. When the procedure is run with 20 μL of this solution so obtained under the above operating conditions, 4-epidemethylchlortetracycline and demethylchlortetracycline are eluted in this order with the resolution between these peaks being not less than 3. The relative retention time of 4-epidemethylchlortetracycline to demethylchlortetracycline is about 0.7.

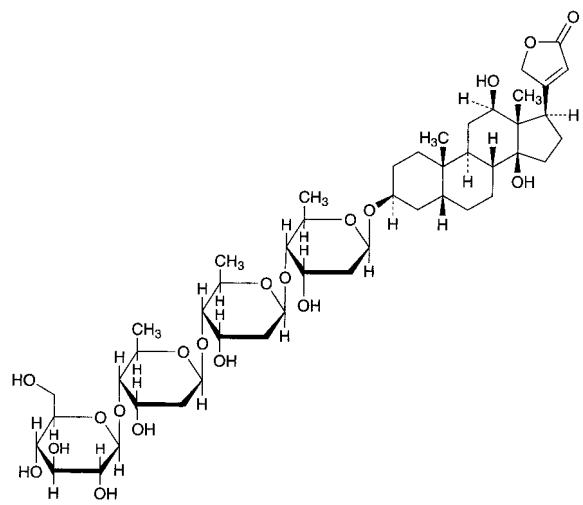
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Deslanoside

デスラノシド



$C_{47}H_{74}O_{19}$: 943.08

3β-[β-D-Glucopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide

[*17598-65-1*]

Deslanoside, when dried, contains not less than 90.0% and not more than 102.0% of deslanoside ($C_{47}H_{74}O_{19}$).

Description Deslanoside occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

It is freely soluble in dehydrated pyridine, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is hygroscopic.

Identification Transfer 1 mg of Deslanoside to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 1000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of two liquids a brown ring is produced, and the color of the upper layer near to the contact zone changes gradually to blue through purple, and the entire acetic acid layer shows a blue-green color through a deep blue color.

Purity **(1)** Clarity and color of solution—Dissolve 20 mg of Deslanoside in 10 mL of ethanol (95) and 3 mL of water by warming, cool, and dilute to 100 mL with water: the solution is clear and colorless.

(2) Related substances—Dissolve 10 mg of Deslanoside in exactly 5 mL of methanol, and use this solution as the sample solution. Dissolve 1.0 mg of Deslanoside RS in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: the spots other than the principal spot obtained from the sample solution are not

larger and not more intense than the spot obtained from the standard solution.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +6.5 – +8.5° (after drying, 0.5 g, dehydrated pyridine, 25 mL, 100 mm).

Loss on drying ⟨*2.41*⟩ Not more than 8.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (0.1 g).

Assay Dissolve about 12 mg each of Deslanoside and Deslanoside RS, previously dried and accurately weighed, in 20 mL each of methanol, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of these solutions, transfer to light-resistant, 25-mL volumetric flasks, shake well with 5 mL each of 2,4,6-trinitrophenol TS and 0.5 mL each of a solution of sodium hydroxide (1 in 10), add diluted methanol (1 in 4) to make 25 mL, and allow to stand at a temperature between 18°C and 22°C for 25 minutes. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution, respectively, at 485 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution prepared with 5 mL of diluted methanol (1 in 5) in the same manner as the blank.

Amount (mg) of deslanoside ($C_{47}H_{74}O_{19}$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Deslanoside RS taken

Containers and storage Containers—Tight containers.

Deslanoside Injection

デスラノシド注射液

Deslanoside Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of deslanoside ($C_{47}H_{74}O_{19}$: 943.08).

Method of preparation Dissolve Deslanoside in 10 vol% ethanol and prepare as directed under Injections. It may contain Glycerin. It may be prepared with a suitable amount of Ethanol and Water for Injection or Sterile Water for Injection in Containers.

Description Deslanoside Injection is a clear and colorless liquid.

pH: 5.0 – 7.0

Identification **(1)** Place a volume of Deslanoside Injection, equivalent to 2 mg of Deslanoside, in a separator, add sodium chloride in the ratio of 0.2 g to each mL of this solution, and extract with three 10-mL portions of chloroform. Combine the chloroform extracts, mix uniformly, pipet 15 mL of this solution, and evaporate the chloroform under reduced pressure. Proceed with the residue as directed in the Identification under Deslanoside.

(2) Evaporate the remaining chloroform extract obtained in (1) under reduced pressure, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Deslanoside RS in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat the plate at 110°C for 10 minutes: the spots from the sample solution and standard solution show a black color and have the same Rf value.

Bacterial endotoxins ⟨*4.01*⟩ Less than 500 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Deslanoside Injection, equivalent to about 3 mg of deslanoside ($C_{47}H_{74}O_{19}$). Add 5 mL of methanol and water to make exactly 25 mL. Use this solution as the sample solution, and proceed as directed in the Assay under Deslanoside.

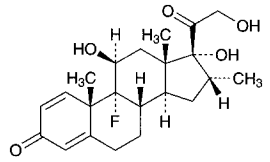
Amount (mg) of deslanoside ($C_{47}H_{74}O_{19}$) = $M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of Deslanoside RS taken

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Dexamethasone

デキサメタゾン



$C_{22}H_{29}FO_5$: 392.46

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione

[*50-02-2*]

Dexamethasone, when dried, contains not less than 97.0% and not more than 102.0% of dexamethasone ($C_{22}H_{29}FO_5$).

Description Dexamethasone occurs as white to pale yellow, crystals or crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in acetonitrile, and practically insoluble in water.

Melting point: about 245°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Proceed with 10 mg of Dexamethasone as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution obtained responds to the Qualitative Tests ⟨*1.09*⟩ for fluoride.

(2) Dissolve 1 mg of Dexamethasone in 10 mL of ethanol (95). Mix 2 mL of the solution with 10 mL of phenylhydrazinium chloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible

Spectrophotometry <2.24>, using as the blank the solution prepared with 2 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dexamethasone RS prepared in the same manner as the former solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Dexamethasone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Dexamethasone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Dexamethasone and Dexamethasone RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +86 – +94° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Dexamethasone according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.18 g of Dexamethasone in 100 mL of acetonitrile. To 33 mL of this solution add a solution, prepared by dissolving 1.32 g of ammonium formate in water to make 1000 mL and adjusted to pH 3.6 with formic acid, to make 100 mL, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than dexamethasone obtained from the sample solution is not larger than the peak area of dexamethasone obtained from the standard solution, and the total area of the peaks other than dexamethasone from the sample solution is not larger than 2 times the peak area of dexamethasone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.32 g of ammonium formate in 1000 mL of water, and adjust the pH to 3.6 with formic acid. To 670 mL of this solution add 330 mL of acetonitrile.

Flow rate: Adjust so that the retention time of dexamethasone is about 13 minutes.

Time span of measurement: About 4 times as long as the retention time of dexamethasone, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of dexamethasone obtained with 10 μL of this solution is equivalent to 8 to 12% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dexamethasone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dexamethasone is not more than 1.0%.

Loss on drying <2.41> Not more than 0.5% (0.2 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.2 g, platinum crucible).

Assay Dissolve about 10 mg each of Dexamethasone and Dexamethasone RS, previously dried and accurately weighed, in 70 mL each of diluted methanol (1 in 2), add exactly 5 mL each of the internal standard solution, then add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dexamethasone to that of the internal standard.

$$\text{Amount (mg) of dexamethasone } (C_{22}H_{29}FO_5) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Dexamethasone RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (2:1).

Flow rate: Adjust so that the retention time of dexamethasone is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, dexamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dexamethasone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dextran 40

デキストラン **40**

Dextran 40 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 40,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 40.

Description Dextran 40 occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves gradually in water.

It is hygroscopic.

Identification To 1 mL of a solution of Dextran 40 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Dextran 40 in 10 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Dextran 40 in 10 mL of water by warming: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Perform the test with 2.0 g of Dextran 40. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Dextran 40 according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.5 g of Dextran 40 according to Method 1, and perform the test (not more than 1.3 ppm).

(5) Nitrogen—Weigh accurately about 2 g of Dextran 40, previously dried, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩, where 10 mL of sulfuric acid is used for decomposition, and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.01) is not more than 0.010%.

(6) Reducing substances—Weigh exactly 3.00 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.450 g of glucose, previously dried, dissolve in water to make exactly 500 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL each of these solutions, add 5 mL of alkaline copper TS, exactly measured, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodine (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate ⟨*2.50*⟩ with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The titrant consumed for the sample solution is not less than that for the control solution.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (1 g, 105°C, 6 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.5 EU/g.

Viscosity ⟨*2.53*⟩ **(1)** Dextran 40—Weigh accurately 0.2 to 0.5 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 at 25°C : the intrinsic viscosity is between 0.16 and 0.19.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually 80 to 90 mL) at 25 ± 1°C with stirring. Dissolve the precipitate at 35°C in a water bath with occasional shaking, and allow to stand for more than 15 hours at 25 ± 1°C. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not more than 0.27.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 115 to 135 mL) at 25 ± 1°C with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath. Dry the residue, and determined the intrinsic viscosity of the dried substance as directed in (1): the value is not less than 0.09.

Antigenicity Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Assay Weigh accurately about 3 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D with the sample solution as directed under Optical Rotation Determination ⟨*2.49*⟩ in a 100-mL cell at 20 ± 1°C.

$$\text{Amount (mg) of dextran 40} = \alpha_D \times 253.8$$

Containers and storage Containers—Tight containers.

Dextran 40 Injection

デキストラン **40** 注射液

Dextran 40 Injection is an aqueous injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of dextran 40.

Method of preparation

Dextran 40	10 g
Isotonic Sodium Chloride Solution	a sufficient quantity
	To make 100 mL

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description Dextran 40 Injection is a clear and colorless liquid. It is slightly viscous.

Identification **(1)** Dilute 1 mL of Dextran 40 Injection with water to 200 mL, and to 1 mL of the diluted solution add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution does not change in color.

(2) Dextran 40 Injection responds to the Qualitative Tests <*1.09*> for sodium salt and for chloride.

pH <*2.54*> 4.5 – 7.0

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Viscosity <*2.53*> Measure exactly 2 to 5 mL of Dextran 40 Injection, add isotonic sodiumchloride solution to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with isotonic sodium chloride solution as directed in Method 1 at 25°C: the intrinsic viscosity is between 0.16 and 0.19. Calculate the concentration of the sample solution (g/100 mL) as directed in the Assay.

Assay To exactly 30 mL of Dextran 40 Injection add water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D with the sample solution as directed under Optical Rotation Determination <*2.49*> in a 100-mm cell at 20 ± 1°C.

Amount (mg) of dextran 40 in 100 mL of Dextran 40 Injection
$= \alpha_D \times 846.0$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Avoid exposure to undue fluctuations in temperature.

Dextran 70

デキストラン **70**

Dextran 70 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 70,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 70.

Description Dextran 70 occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves gradually in water.

It is hygroscopic.

Identification To 1 mL of a solution of Dextran 70 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

pH <*2.54*> Dissolve 3.0 g of Dextran 70 in 50 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Dextran 70 in 10 mL of water with warming: the solution is clear and colorless.

(2) Chloride <*1.03*>—With 2.0 g of Dextran 70, perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Dextran 70 according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.5 g of Dextran 70 according to Method 1, and perform the test (not more than 1.3 ppm).

(5) Nitrogen—Weigh accurately about 2 g of Dextran 70, previously dried, perform the test as directed under Nitrogen Determination <*1.08*>, where 10 mL of sulfuric acid is used for decomposition, and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.007) is not more than 0.010%.

(6) Reducing substances—Weigh exactly 3.00 g of Dextran 70, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.300 g of glucose, previously dried, dissolve in water to make exactly 500 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL of these diluted solutions, add exactly 5 mL of alkaline copper TS, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodide (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate <*2.50*> with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The titrant consumed for the sample solution is not less than that for the control solution.

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 6 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Viscosity <*2.53*> **(1)** Dextran 70—Weigh accurately 0.2 to 0.5 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 at 25°C : the intrinsic viscosity is between 0.21 and 0.26.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually, 75 to 85 mL) at 25 ± 1°C with stirring. Dissolve the precipitate in a water bath at 35°C with occasional shaking, and allow to stand for more than 15 hours at 25 ± 1°C. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer on a water bath to dryness. Dry the residue, and determine the intrinsic viscosity of the dried residue as directed in (1): the value is not more than 0.35.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 110 to 130 mL) at 25 ± 1°C with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried residue as directed in (1): the value is not less than 0.10.

Antigenicity Dissolve 6.0 g of Dextran 70 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Separately, inject 0.10 mL of

horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pigs of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Pyrogen ⟨*4.04*⟩ Dissolve 6.0 g of Dextran 70 in isotonic sodium chloride solution to make 100 mL, and perform the test: this solution meets the requirement.

Assay Weigh accurately about 3 g of Dextran 70, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D as directed under Optical Rotation Determination ⟨*2.49*⟩ in a 100-mm cell at 20 ± 1°C.

$$\text{Amount (mg) of dextran 70} \times \alpha_D = 253.8$$

Containers and storage Containers—Tight containers.

Dextran Sulfate Sodium Sulfur 5

デキストラン硫酸エステルナトリウム イオウ 5

Dextran Sulfate Sodium Sulfur 5 is a sodium salt of sulfate ester obtained by sulfation of partial decomposition products of dextran, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* Van Tieghem (*Lactobacillaceae*).

Description Dextran Sulfate Sodium Sulfur 5 occurs as a white to light yellowish white powder. It is odorless, and has a saline taste.

It is freely soluble in water and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification **(1)** To 10 mL of a solution of toluidine blue (1 in 100,000) add 0.05 mL of a solution of Dextran Sulfate Sodium Sulfur 5 (3 in 50) dropwise: a color of the solution changes from blue to red-purple.

(2) To 1 mL of a solution of Dextran Sulfate Sodium Sulfur 5 (1 in 1500) add 2 mL of anthrone TS: a blue-green color develops, which turns dark blue-green gradually. Then, add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution remains dark blue-green.

(3) A solution of Dextran Sulfate Sodium Sulfur 5 (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +135.0 – +155.0° (1.5 g calculated on the dried basis, water, 25 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Dextran Sulfate Sodium Sulfur 5 in 20 mL of water: the pH of this solution is between 5.5 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 2.5 g of Dextran Sulfate Sodium Sulfur 5 in 50 mL of water: the solution is clear. And, determine the absorbance of the solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: not more than 0.090.

(2) Chloride ⟨*1.03*⟩—Perform the test with 0.10 g of Dextran Sulfate Sodium Sulfur 5. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.106%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.10 g of Dextran Sulfate Sodium Sulfur 5 in 6 mL of water, add 0.6 mL of barium chloride TS, and heat in a water bath for 4 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL, allow to stand for 10 minutes, and observe: the turbidity of the solution is not more intense than that of the control solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 6 mL of water, and proceed in the same manner (not more than 0.240%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Dextran Sulfate Sodium Sulfur 5 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Dextran Sulfate Sodium Sulfur 5 according to Method 3, and perform the test (not more than 2 ppm).

Sulfur content Weigh accurately about 1.0 g of Dextran Sulfate Sodium Sulfur 5, dissolve in 5 mL of water, add 1.5 mL of hydrochloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. To exactly 10 mL of the sample solution add exactly 20 mL of 0.02 mol/L barium chloride VS, add 5 mL of methanol, and heat in a water bath for 30 minutes. After cooling, neutralize with sodium hydroxide TS, and add 70 mL of water, 10 mL of a solution of zinc disodium ethylenediamine tetraacetate tetrahydrate (1 in 20), 3 mL of ammonium chloride TS and 7 mL of strong ammonium water, and titrate ⟨*2.50*⟩ with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to light blue (indicator: 5 drops of eriochrome black T TS). Perform a blank determination. Amount of sulfur (S: 32.07), calculated on the dried basis, is between 3.0 and 6.0%.

$$\text{Each mL of 0.02 mol/L barium chloride VS} = 0.6414 \text{ mg of S}$$

Loss on drying ⟨*2.41*⟩ Not more than 10.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Viscosity ⟨*2.53*⟩ Weigh accurately about 1.5 g of Dextran Sulfate Sodium Sulfur 5, calculated on the dried basis, dissolve in a solution of sodium chloride (29 in 500) to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and a solution of sodium chloride (29 in 500) at 25 ± 0.02°C as directed: the intrinsic viscosity is between 0.030 and 0.040.

Containers and storage Containers—Tight containers.

Dextran Sulfate Sodium Sulfur 18

デキストラン硫酸エステルナトリウム イオウ 18

Dextran Sulfate Sodium Sulfur 18 is a sodium salt of sulfate ester obtained by sulfation of partial decomposition products of dextran, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* Van Tieghem (*Lactobacillaceae*).

Description Dextran Sulfate Sodium Sulfur 18 occurs as a

white to light yellowish white powder. It is odorless, and has a saline taste.

It is freely soluble in water and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification **(1)** To 10 mL of a solution of toluidine blue (1 in 100,000) add 0.05 mL of a solution of Dextran Sulfate Sodium Sulfur 18 (3 in 50) dropwise: a color of the solution changes from blue to red-purple.

(2) To 1 mL of a solution of Dextran Sulfate Sodium Sulfur 18 (1 in 1500) add 2 mL of anthrone TS: a blue-green color develops, which turns dark blue-green gradually. Then, add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution remains dark blue-green.

(3) A solution of Dextran Sulfate Sodium Sulfur 18 (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +90.0 – +110.0° (1.5 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Dextran Sulfate Sodium Sulfur 18 in 20 mL of water: the pH of this solution is between 5.5 and 7.5.

Purity **(1)** Chloride <*1.03*>—Perform the test with 0.10 g of Dextran Sulfate Sodium Sulfer 18. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.106%).

(2) Sulfate <*1.14*>—Dissolve 0.10 g of Dextran Sulfate Sodium Sulfur 18 in 6 mL of water, add 0.6 mL of barium chloride TS, and heat in a water bath for 4 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL, allow to stand for 10 minutes, and observe: the turbidity of the solution is not more intense than that of the control solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS add 6 mL of water, and proceed in the same manner (not more than 0.480%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Dextran Sulfate Sodium Sulfur 18 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Dextran Sulfate Sodium Sulfur 18 according to Method 3, and perform the test (not more than 2 ppm).

Sulfur content Weigh accurately about 0.5 g of Dextran Sulfate Sodium Sulfur 18, dissolve in 5 mL of water, add 1.5 mL of hydrochloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. To exactly 10 mL of the sample solution add exactly 20 mL of 0.02 mol/L barium chloride VS, add 5 mL of methanol, and heat in a water bath for 30 minutes. After cooling, neutralize with sodium hydroxide TS, and add 70 mL of water, 10 mL of a solution of zinc disodium ethylenediamine tetraacetate tetrahydrate (1 in 20), 3 mL of ammonium chloride TS and 7 mL of strong ammonium water, and titrate <*2.50*> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to light blue (indicator: 5 drops of eriochrome black T TS). Perform a blank determination. Amount of sulfur (S: 32.07), calculated on the dried basis, is between 15.0 and 20.0%.

Each mL of 0.02 mol/L barium chloride VS
= 0.6414 mg of S

Loss on drying <*2.41*> Not more than 10.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Viscosity <*2.53*> Weigh accurately about 1.5 g of Dextran Sulfate Sodium Sulfur 18, calculated on the dried basis, dissolve in a solution of sodium chloride (29 in 500) to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and a solution of sodium chloride (29 in 500) at 25 ± 0.02°C as directed: the intrinsic viscosity is between 0.020 and 0.032.

Containers and storage Containers—Tight containers.

Dextrin

デキストリン

Description Dextrin occurs as a white or light yellow, amorphous powder or granules. It has a slight, characteristic odor and a sweet taste. It does not irritate the tongue.

Dextrin is freely soluble in boiling water, soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification To 0.1 g of Dextrin add 100 mL of water, shake, and filter if necessary. To 5 mL of the filtrate add 1 drop of iodine TS: a light red-brown or light red-purple color develops.

Purity **(1)** Clarity and color of solution—Take 2.0 g of Dextrin in a Nessler tube, add 40 mL of water, dissolve by heating, cool, and add water to make 50 mL: the solution is colorless or light yellow. It is clear, and even if turbid, the turbidity is not more than that of the following control solution.

Control solution: To 1.0 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 46 mL of water and 2 mL of barium chloride TS, allow to stand for 10 minutes, and shake before use.

(2) Acidity—To 1.0 g of Dextrin add 5 mL of water, dissolve by heating, cool, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <*1.03*>—To 2.0 g of Dextrin add 80 mL of water, dissolve by heating, cool, add water to make 100 mL, and filter. Take 40 mL of the filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.013%).

(4) Sulfate <*1.14*>—To 45 mL of the filtrate obtained in (3) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(5) Oxalate—To 1.0 g of Dextrin add 20 mL of water, dissolve by heating, cool, add 1 mL of acetic acid (31), and filter. To 5 mL of the filtrate add 5 drops of calcium chloride TS: no turbidity is produced immediately.

(6) Calcium—To a 5-mL portion of the filtrate obtained in (5) add 5 drops of ammonium oxalate TS: no turbidity is immediately produced.

(7) Heavy metals <*1.07*>—Proceed with 0.5 g of Dextrin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

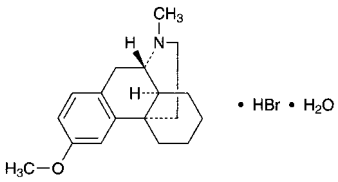
Loss on drying <*2.41*> Not more than 10% (0.5 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (0.5 g).

Containers and storage Containers—Well-closed containers.

Dextromethorphan Hydrobromide Hydrate

デキストロメトルファン臭化水素酸塩水和物



$C_{18}H_{25}NO.HBr.H_2O$: 370.32
(9*S*,13*S*,14*S*)-3-Methoxy-17-methylmorphinan monohydrobromide monohydrate
[*6700-34-1*]

Dextromethorphan Hydrobromide Hydrate contains not less than 98.0% of dextromethorphan hydrobromide ($C_{18}H_{25}NO.HBr$: 352.31), calculated on the anhydrous basis.

Description Dextromethorphan Hydrobromide Hydrate occurs as white, crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95) and in acetic acid (100), and sparingly soluble in water.

Melting point: about 126°C (Insert the capillary tube into the bath preheated to 116°C, and continue the heating so that the temperature rises at a rate of about 3°C per minute.)

Identification **(1)** Determine the absorption spectrum of a solution of Dextromethorphan Hydrobromide Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dextromethorphan Hydrobromide Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 50 mL of a solution of Dextromethorphan Hydrobromide Hydrate (1 in 100) add 2 drops of phenolphalein TS and sodium hydroxide TS until a red color develops. Add 50 mL of chloroform, shake, and add 5 mL of dilute nitric acid to 40 mL of the water layer. This solution responds to the Qualitative Tests ⟨*1.09*⟩ for bromide.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +26 – +30° (0.34 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Dextromethorphan Hydrobromide Hydrate in 100 mL of water: the pH of this solution is between 5.2 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Dextromethorphan Hydrobromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) *N*,*N*-dimethylaniline—To 0.50 g of Dextromethorphan Hydrobromide Hydrate add 20 mL of water, and dissolve by heating on a water bath. After cooling, add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL: the solution has no more color than the following control solution.

Control solution: Dissolve 0.10 g of *N*,*N*-dimethylaniline in 400 mL of water by warming on a water bath, cool, and add water to make 500 mL. Pipet 5 mL of this solution, and add water to make 200 mL. To 1.0 mL of this solution add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Dextromethorphan Hydrobromide Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Phenolic compounds—Dissolve 5 mg of Dextromethorphan Hydrobromide Hydrate in 1 drop of dilute hydrochloric acid and 1 mL of water, add 2 drops of iron (III) chloride TS and 2 drops of potassium hexacyanoferrate (III) TS, shake, and allow to stand for 15 minutes: no blue-green color develops.

(5) Related substances—Dissolve 0.25 g of Dextromethorphan Hydrobromide Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, ethyl acetate, methanol, dichloromethane and 13.5 mol/L ammonia TS (55:20:13:10:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly bismuth potassium iodide TS on the plate, and then spray evenly hydrogen peroxide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ 4.0 – 5.5% (0.2 g, volumetric titration, back titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Dextromethorphan Hydrobromide Hydrate, dissolve in 10 mL of acetic acid (100) and add 40 mL of acetic anhydride. Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.23 mg of $C_{18}H_{25}NO.HBr$

Containers and storage Containers—Well-closed containers.

Diastase

ジアスターゼ

Diastase is an enzyme drug mainly prepared from malt. It has amylolytic activity.

It contains not less than 440 starch saccharifying activity units per g.

It is usually diluted with suitable diluents.

Description Diastase occurs as a light yellow to light brown powder.

It is hygroscopic.

Purity Rancidity—Diastase has no unpleasant or rancid

odor, and has no unpleasant or rancid taste.

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (1 g, 105°C, 5 hours).

Assay (i) Substrate solution—Use potato starch TS for amylolytic activity test.

(ii) Sample solution—Weigh accurately about 0.1 g of Diastase, and dissolve in water to make exactly 100 mL.

(iii) Procedure—Proceed as directed in 1.1. Measurement of starch saccharifying activity of 1. Assay for starch digestive activity under Digestion Test ⟨*4.03*⟩.

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Diastase and Sodium Bicarbonate Powder

ジアスターゼ·重曹散

Method of preparation

Diastase	200 g
Sodium Bicarbonate	300 g
Precipitated Calcium Carbonate	400 g
Magnesium Oxide	100 g
To make	1000 g

Prepare before use as directed under Powders, with the above ingredients.

Description Diastase and Sodium Bicarbonate Powder occurs as a light yellow powder. It has a characteristic, salty taste.

Containers and storage Containers—Well-closed containers.

Compound Diastase and Sodium Bicarbonate Powder

複方ジアスターゼ·重曹散

Method of preparation

Diastase	200 g
Sodium Bicarbonate	600 g
Magnesium Oxide	150 g
Powdered Gentian	50 g
To make	1000 g

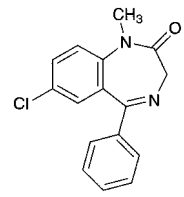
Prepare before use as directed under Powders, with the above ingredients.

Description Compound Diastase and Sodium Bicarbonate Powder occurs as a slightly brownish, light yellow powder. It has a characteristic odor and a bitter taste.

Containers and storage Containers—Well-closed containers.

Diazepam

ジアゼパム



$C_{16}H_{13}ClN_2O$: 284.74

7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one

[*439-14-5*]

Diazepam, when dried, contains not less than 98.0% of diazepam ($C_{16}H_{13}ClN_2O$).

Description Diazepam occurs as a white to light yellow crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone, soluble in acetic anhydride and in ethanol (95), sparingly soluble in diethyl ether, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Dissolve 10 mg of Diazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(2) Dissolve 2 mg of Diazepam in 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Diazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Diazepam as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a blue to blue-green color appears.

Melting point ⟨*2.60*⟩ 130 – 134°C

Purity **(1)** Clarity of solution—Dissolve 0.10 g of Diazepam in 20 mL of ethanol (95): the solution is clear.

(2) Chloride ⟨*1.03*⟩—To 1.0 g of Diazepam add 50 mL of water, allow to stand for 1 hour, with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Diazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 1.0 g of Diazepam in 10 mL of acetone, and use this solution as the sample solution. Pipept 1 mL of the sample solution, and add acetone to make exactly 100 mL. Pipet 1 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as

directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Diazepam, previously dried, dissolve in 60 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.47 mg of $C_{16}H_{13}ClN_2O$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Diazepam Tablets

ジアゼパム錠

Diazepam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of diazepam ($C_{16}H_{13}ClN_2O$: 284.74).

Method of preparation Prepare as directed under Tablets, with Diazepam.

Identification To a portion of the powdered Diazepam Tablets, equivalent to 50 mg of Diazepam, add 50 mL of acetone, shake, and filter. Evaporate 1 mL of the filtrate on a water bath to dryness, and dissolve the residue with 100 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 240 nm and 244 nm, between 283 nm and 287 nm, and between 360 nm and 370 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Diazepam Tablets add 5 mL of water, and disintegrate the tablet by shaking. Then add 30 mL of methanol, shake for 10 minutes, add methanol to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to 0.4 mg of diazepam ($C_{16}H_{13}ClN_2O$), add exactly 5 mL of the internal standard solution, then add methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of diazepam for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and add methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ under the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diazepam to that of the internal standard.

Amount (mg) of diazepam ($C_{16}H_{13}ClN_2O$)
$= M_S \times Q_T/Q_S \times 1/V$

M_S: Amount (mg) of diazepam for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 25,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and diazepam are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diazepam to that of the internal standard is not more than 1.0%.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately the mass of not less than 20 Diazepam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of diazepam ($C_{16}H_{13}ClN_2O$), add 10 mL of water, shake, then add 60 mL of methanol, shake for 10 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of diazepam for assay, previously dried at 105°C for 2 hours, and dissolve in 10 mL of water and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diazepam to that of the internal standard.

Amount (mg) of diazepam ($C_{16}H_{13}ClN_2O$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of diazepam for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and water (13:7).

Flow rate: Adjust so that the retention time of diazepam is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and diazepam are eluted in this order with the resolution between these peaks being not more than 6.

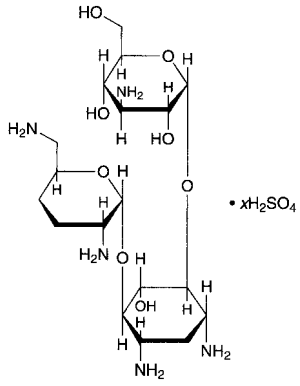
System repeatability: When the test is repeated 6 times

with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diazepam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Dibekacin Sulfate

ジベカシン硫酸塩



$C_{18}H_{37}N_5O_8.xH_2SO_4$

3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranosyl-(1→4)]-2-deoxy-D-streptamine sulfate

[*58580-55-5*]

Dibekacin Sulfate is the sulfate of a derivative of bekanamycin.

It contains not less than 640 μg (potency) and not more than 740 μg (potency) per mg, calculated on the dried basis. The potency of Dibekacin Sulfate is expressed as mass (potency) of dibekacin ($C_{18}H_{37}N_5O_8$: 451.52).

Description Dibekacin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 20 mg each of Dibekacin Sulfate and Dibekacin Sulfate RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28) and methanol (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the spot obtained from the standard solution show a purple-brown color and the same *R*f value.

(2) To 5 mL of a solution of Dibekacin Sulfate (1 in 50) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +96 – +106° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Dibekacin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 3.0 g of Dibekacin Sulfate in 10 mL of water: the solution is clear. Determine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: not more than 0.15.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Dibekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <*2.41*> Not more than 5.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer having pH 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Dibekacin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution (pH 6.0) (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Dibekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Dibekacin Sulfate Ophthalmic Solution

ジベカシン硫酸塩点眼液

Dibekacin Sulfate Ophthalmic Solution is an aqueous ophthalmic preparation.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of dibekacin ($C_{18}H_{37}N_5O_8$: 451.52).

Method of preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Dibekacin Sulfate.

Description Dibekacin Sulfate Ophthalmic Solution is a clear, colorless liquid.

Identification To a volume of Dibekacin Sulfate Ophthalmic Solution add water so that each mL contains about 2.5 mg (potency) of Dibekacin Sulfate, and use this solution as the sample solution. Separately, dissolve an amount of Dibekacin Sulfate RS, equivalent to 5 mg (potency), in 2 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for

thin-layer chromatography. Proceed as directed in the Identification (1) under Dibekacin Sulfate.

pH ⟨*2.54*⟩ 6.5 – 7.5

Foreign insoluble matter ⟨*6.11*⟩ It meets the requirement.

Insoluble particulate matter ⟨*6.08*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, culture medium and standard solutions—Proceed as directed in the Assay under Dibekacin Sulfate.

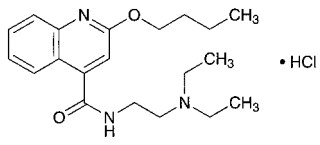
(ii) Sample solutions—Pipet a volume of Dibekacin Sulfate Ophthalmic Solution, equivalent to about 12 mg (potency), and add water to make exactly 30 mL. Pipet a suitable volume of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Dibucaine Hydrochloride

Cinchocaine Hydrochloride

ジブカイン塩酸塩



$C_{20}H_{29}N_3O_2.HCl$: 379.92
2-Butyloxy-*N*-(2-diethylaminoethyl)-4-quinolinecarboxamide monohydrochloride
[*61-12-1*]

Dibucaine Hydrochloride, when dried, contains not less than 98.0% of dibucaine hydrochloride ($C_{20}H_{29}N_3O_2.HCl$).

Description Dibucaine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, in ethanol (95) and in acetic acid (100), freely soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Dibucaine Hydrochloride in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dibucaine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dibucaine Hydrochloride (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Dibucaine Hydrochloride in 50 mL of water: the pH of this solution is between 5.0 and 6.0.

Melting point ⟨*2.60*⟩ 95 – 100°C Charge Dibucaine Hydrochloride into a capillary tube for melting point determination, and dry in vacuum over phosphorus (V) oxide at 80°C for 5 hours. Seal immediately the open end of the tube, and determine the melting point.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Dibucaine Hydrochloride in 20 mL of water: the solution is clear and colorless. Determine the absorbance of this solution at 430 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank: it is not more than 0.03.

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.30 g of Dibucaine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.056%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Dibucaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.20 g of Dibucaine Hydrochloride in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL, then pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

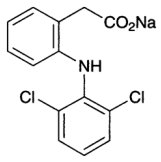
Assay Weigh accurately about 0.3 g of Dibucaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.00 mg of $C_{20}H_{29}N_3O_2.HCl$

Containers and storage Containers—Tight containers.

Diclofenac Sodium

ジクロフェナクナトリウム



$C_{14}H_{10}Cl_2NNaO_2$: 318.13
Monosodium 2-(2,6-dichlorophenylamino)phenylacetate
[*15307-79-6*]

Diclofenac Sodium, when dried, contains not less than 98.5% of dichlofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$).

Description Diclofenac Sodium occurs as white to pale yellowish white, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), sparingly soluble in water and in acetic acid (100), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** To 1 mL of a solution of Diclofenac Sodium in methanol (1 in 250) add 1 mL of nitric acid: a dark red color develops.

(2) Perform the test with 5 mg of Diclofenac Sodium as directed under Flame Coloration Test <*1.04*> (2): a light green color appears.

(3) Determine the infrared absorption spectrum of Diclofenac Sodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Diclofenac Sodium (1 in 100) responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Diclofenac Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Diclofenac Sodium according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Diclofenac Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of each peak other than diclofenac obtained from the sample solution is not larger than the peak area of diclofenac obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (3 in 2500) (4:3).

Flow rate: Adjust so that the retention time of diclofenac is about 20 minutes.

Time span of measurement: About twice as long as the retention time of diclofenac, beginning after the solvent peak.

System suitability—

System performance: Dissolve 35 mg of ethyl parahydroxybenzoate and 0.05 g of propyl parahydroxybenzoate in 100 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, ethyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of diclofenac is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.5 g of Diclofenac Sodium, previously dried, dissolve in 40 mL of water in a separator, add 2 mL of dilute hydrochloric acid, and extract the precipitate formed with 50 mL of chloroform. Extract again with two 20-mL portions of chloroform, and filter the extract each time through a pledget of absorbent cotton moistened with chloroform. Wash the tip of the separator and the absorbent cotton with 15 mL of chloroform, combine the washing with the extracts, add 10 mL of a solution of 1 mol/L hydrochloric acid TS in ethanol (99.5) (1 in 100), and titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS from the first equivalent point to the second equivalent point (potentiometric titration).

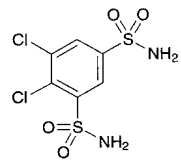
Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 31.81 mg of $C_{14}H_{10}Cl_2NNaO_2$

Containers and storage Containers—Tight containers.

Diclofenamide

Dichlorphenamide

ジクロフェナミド



$C_6H_6Cl_2N_2O_4S_2$: 305.16
4,5-Dichlorobenzene-1,3-disulfonamide
[*120-97-8*]

Diclofenamide, when dried, contains not less than 98.0% of dichlofenamide ($C_6H_6Cl_2N_2O_4S_2$).

Description Diclofenamide occurs as a white crystalline powder.

It is very soluble in *N*,*N*-dimethylformamide, soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 0.01 g of Diclofenamide in 100

mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of the solution add 0.1 mL of hydrochloric acid. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Diclofenamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Diclofenamide, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Diclofenamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 237 – 240°C

Purity **(1)** Chloride <*1.03*>—Dissolve 0.10 g of Diclofenamide in 10 mL of *N*,*N*-dimethylformamide, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of *N*,*N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.160%).

(2) Selenium—To 0.10 g of Diclofenamide add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 2 mL of nitric acid, and heat on a water bath until no more brown gas evolves and the solution becomes to be a light yellow clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 3 mL of Standard Selenium Solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 6 mL of nitric acid, then add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine constant absorbances, A_T and A_S, obtained on a recorder after rapid increasing of the absorption: A_T is smaller than A_S (not more than 30 ppm).

Perform the test by using a hydride generating system and a thermal absorption cell.

Lamp: A selenium hollow cathode lamp.

Wavelength: 196.0 nm.

Temperature of sample atomizer: When an electric furnace is used, about 1000°C.

Carrier gas: Nitrogen or Argon.

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Diclofenamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Diclofenamide in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than diclofenamide obtained from the sample solution is not larger than the peak area of diclofenamide obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of diclofenamide.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of diclofenamide obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diclofenamide is not more than 1.0%.

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 5 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Diclofenamide and Diclofenamide RS, previously dried, and dissolve each in 30 mL of the mobile phase. To each add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diclofenamide to that of the internal standard.

$$\text{Amount (mg) of dichlofenamide } (C_6H_6Cl_2N_2O_4S_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Diclofenamide RS taken

Internal standard solution—A solution of butyl parahydroxy benzoate in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of sodium phosphate TS and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of diclofenamide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, diclofenamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of diclofenamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Diclofenamide Tablets

Dichlorphenamide Tablets

ジクロフェナミド錠

Diclofenamide Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$: 305.16).

Method of preparation Prepare as directed under Tablets, with Diclofenamide.

Identification To a quantity of powdered Diclofenamide Tablets, equivalent to 0.2 g of Diclofenamide, add 20 mL of methanol, shake, and filter. Evaporate the filtrate on a water bath to dryness, and dissolve 0.01 g of the residue in 100 mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of this solution add 0.1 mL of hydrochloric acid TS, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 284 nm and 288 nm, and between 293 nm and 297 nm.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Diclofenamide Tablets is not less than 70%.

Start the test with 1 tablet of Diclofenamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$), and use this solution as the sample solution. Separately, weigh accurately about 55 mg of Diclofenamide RS, previously dried under reduced pressure not exceeding 0.67 kPa at 100°C for 5 hours, dissolve in 10 mL of ethanol (95), and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Diclofenamide RS taken
C: Labeled amount (mg) of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 tablets of Diclofenamide Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$), add exactly 25 mL of the mobile phase, shake for 15 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Diclofenamide RS, previously dried in vacuum at a pressure not exceeding 0.67 kPa at 100°C for 5 hours, dissolve in 30 mL of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Diclofenamide.

Amount (mg) of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$)
$= M_S \times Q_T/Q_S$

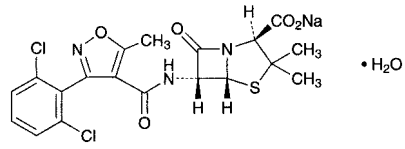
M_S: Amount (mg) of Diclofenamide RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (3 in 5000).

Containers and storage Containers—Well-closed containers.

Dicloxacillin Sodium Hydrate

ジクロキサシリンナトリウム水和物



$C_{19}H_{16}Cl_2N_3NaO_5S.H_2O$: 510.32
Monosodium (2*S*,5*R*,6*R*)-6-{[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbonyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate
[*13412-64-1*]

Dicloxacillin Sodium Hydrate contains not less than 910 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Dicloxacillin Sodium Hydrate is expressed as mass (potency) of dicroxacillin ($C_{19}H_{17}Cl_2N_3O_5S$: 470.33).

Description Dicloxacillin Sodium Hydrate occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water and in methanol, and soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Dicloxacillin Sodium Hydrate (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dicloxacillin Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dicloxacillin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Dicloxacillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dicloxacillin Sodium Hydrate responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Water <*2.48*> Not less than 3.0% and not more than 4.5% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.5 to 6.6 after sterilization.

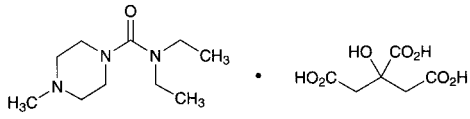
(iii) Standard solutions—Weigh accurately an amount of Dicloxacillin Sodium RS equivalent to about 50 mg (potency), dissolve in phosphate buffer solution (pH 6.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 10 μg (potency) and 2.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Dicloxacillin Sodium Hydrate equivalent to about 50 mg (potency), dissolve in phosphate buffer solution (pH 6.0) to make exactly 50 mL. Take exactly a suitable amount of the solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 10 μg (potency) and 2.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Diethylcarbamazine Citrate

ジエチルカルバマジンクエン酸塩



$C_{10}H_{21}N_3O.C_6H_8O_7$: 391.42
N,*N*-Diethyl-4-methylpiperazine-1-carboxamide monocitrate
[*1642-54-2*]

Diethylcarbamazine Citrate, when dried, contains not less than 98.0% of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$).

Description Diethylcarbamazine Citrate occurs as a white, crystalline powder. It is odorless, and has an acid and bitter taste.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in acetone, in chloroform and in diethyl ether.

A solution of Diethylcarbamazine Citrate (1 in 20) is acid.

Diethylcarbamazine Citrate is hygroscopic.

Identification **(1)** Dissolve 0.5 g of Diethylcarbamazine Citrate in 2 mL of water, add 10 mL of sodium hydroxide TS, and extract with four 5-mL portions of chloroform. Wash the combined chloroform extracts with 10 mL of water, and evaporate the chloroform on a water bath. Add 1 mL of iodoethane to the residue, and boil gently under a reflux condenser for 5 minutes. Evaporate the excess iodoethane with the aid of a current of air, and dissolve the residue in 4 mL of ethanol (95). Cool the ethanol solution in an ice bath, with continuous stirring, add diethyl ether until precipitates are formed, and stir until crystallization is evident. Allow to stand in the ice bath for 30 minutes, and collect the precipitate. Dissolve the precipitate in 4 mL of ethanol (95), repeat the recrystallization in the same manner, then dry at 105°C for 4 hours: the crystals so obtained melt ⟨*2.60*⟩ between 151°C and 155°C.

(2) Neutralize the remaining aqueous layer obtained in (1) with dilute sulfuric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) and (3) for citrate.

Melting point ⟨*2.60*⟩ 135.5 – 138.5°C

Purity Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Diethylcarbamazine Citrate according to Method 4, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (2 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.75 g of Diethylcarbamazine Citrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.14 mg of $C_{10}H_{21}N_3O.C_6H_8O_7$

Containers and storage Containers—Tight containers.

Diethylcarbamazine Citrate Tablets

ジエチルカルバマジンクエン酸塩錠

Diethylcarbamazine Citrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$: 391.42).

Method of preparation Prepare as directed under Tablets, with Diethylcarbamazine Citrate.

Identification To a quantity of the powdered Diethylcarbamazine Citrate Tablets, equivalent to 0.1 g of Diethylcarbamazine Citrate, add 10 mL of water, shake well, and filter. To the filtrate add 1 mL of Reinecke salt TS: a light red precipitate is formed.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Diethylcarbamazine Citrate Tablets add 70 mL of the mobile phase, shake vigorously for 10 minutes, add the mobile phase to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to about 2.5 mg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$), add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$)
$= M_S \times Q_T/Q_S \times 10/V$

M_S: Amount (mg) of Diethylcarbamazine Citrate RS taken

Internal standard solution—A solution of 2-aminobenzimidazol in the mobile phase (1 in 12,500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Diethylcarbamazine Citrate Tablets is not

less than 80%.

Start the test with 1 tablet of Diethylcarbamazine Citrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Diethylcarbamazine Citrate RS, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of diethylcarbamazine in each solution.

Dissolution rate (%) with respect to the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 225$

M_S: Amount (mg) of Diethylcarbamazine Citrate RS taken
C: Labeled amount (mg) of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$) in 1 tablet

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of diethylcarbamazine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diethylcarbamazine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Diethylcarbamazine Citrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$), add 70 mL of the mobile phase, shake vigorously for 10 minutes, add the mobile phase to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Diethylcarbamazine Citrate RS, previously dried at 105°C for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diethylcarbamazine to that of the internal standard.

Amount (mg) of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of Diethylcarbamazine Citrate RS taken

Internal standard solution—A solution of 2-aminobenzimidazole in the mobile phase (1 in 12,500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.05 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 2.5. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust so that the retention time of diethylcarbamazine is about 14 minutes.

System suitability—

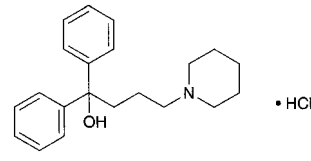
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, diethylcarbamazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diethylcarbamazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Difenidol Hydrochloride

ジフェニドール塩酸塩



$C_{21}H_{27}NO.HCl$: 345.91

1,1-Diphenyl-4-piperidin-1-ylbutan-1-ol monohydrochloride [*3254-89-5*]

Difenidol Hydrochloride, when dried, contains not less than 98.5% of difenidol hydrochloride ($C_{21}H_{27}NO.HCl$).

Description Difenidol Hydrochloride occurs as white, crystals or crystalline powder. It is odorless.

It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in water and in acetic acid (100), and practically insoluble in diethyl ether.

Melting point: about 217°C (with decomposition).

Identification **(1)** Dissolve 0.01 g of Difenidol Hydrochloride in 1 mL of sulfuric acid: an orange-red color develops. To this solution add carefully 3 drops of water: the solution becomes yellowish brown, and colorless on the addition of 10 mL of water.

(2) To 5 mL of a solution of Difenidol Hydrochloride (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

(3) To 10 mL of a solution of Difenidol Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract

with two 15-mL portions of chloroform. Combine the extracts, wash with three 10-mL portions of water, evaporate the chloroform on a water bath, and dry the residue in a desiccator (in vacuum, silica gel, 55°C) for 5 hours: the residue melts ⟨*2.60*⟩ between 103°C and 106°C.

(4) A solution of Difenidol Hydrochloride (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Difenidol Hydrochloride in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.7 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Difenidol Hydrochloride in 10 mL of methanol: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Difenidol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Difenidol Hydrochloride according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.10 g of Difenidol Hydrochloride in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 1,1-diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography in methanol to make exactly 20 mL, pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, methanol and acetic acid (100) (10:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Difenidol Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100) by warming if necessary, cool, add 30 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 17.30 mg of $C_{21}H_{27}NO.HCl$

Containers and storage Containers—Well-closed containers.

Diflorasone Diacetate

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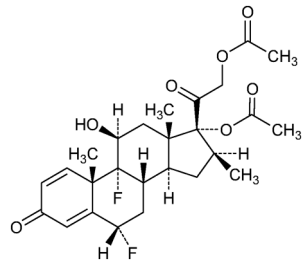


$C_{26}H_{32}F_2O_7$: 494.52

6α,9-Difluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-diacetate

[*33564-31-7*]

Diflorasone Diacetate, when dried, contains not less than 97.0 and not more than 102.0% of diflorasone diacetate ($C_{26}H_{32}F_2O_7$).

Description Diflorasone Diacetate occurs as a white to pale yellow, crystals or crystalline powder.

It is soluble in acetonitrile, slightly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 222°C (with decomposition).

Identification **(1)** Determine the infrared absorption spectrum of Diflorasone Diacetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Diflorasone Diacetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Prepare the test solution with 10 mg of Diflorasone Diacetate as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using 20 mL of diluted 0.01 mol/L sodium hydroxide VS (1 in 40) as the absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ for fluoride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: + 88 – + 93° (after drying, 0.1 g, acetonitrile, 10 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Diflorasone Diacetate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Diflorasone Diacetate in 20 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the areas of the peaks, having a relative retention time of about 0.5, about 0.7, about 0.9 and about 1.1 to diflorasone diacetate, obtained from the sample solution are respectively not larger than 1/4 times, 1/4 times, 1/2 times and 3/4 times the peak area of diflorasone diacetate obtained from the standard solution, and the total area of the peaks other than diflorasone diacetate and the peaks mentioned above from the sample solutions is not larger than 1/5 times the peak area of diflorasone diacetate from the standard solution. Furthermore, the total area of the peaks other than diflorasone diacetate from the sample

solution is not larger than 1.5 times the peak area of diflorasone diacetate from the standard solution.
Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 1.4 times as long as the retention time of diflorasone diacetate, beginning after the solvent peak.
System suitability—
System performance: Proceed as directed in the system suitability in the Assay.
Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of diflorasone diacetate obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution, the relative standard deviation of the peak area of diflorasone diacetate is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.5 g, platinum crucible).

Assay Weigh accurately about 20 mg each of Diflorasone Diacetate and Diflorasone Diacetate RS, both previously dried, dissolve in exactly 4 mL each of the internal standard solution, add acetonitrile to make them 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diflorasone diacetate to that of the internal standard.

$$\text{Amount (mg) of diflorasone diacetate } (C_{26}H_{23}F_2O_7) = M_S \times Q_T/Q_S$$

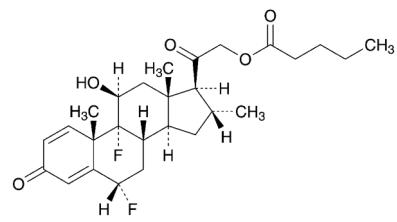
M_S: Amount (mg) of Diflorasone Diacetate RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 200). To 550 mL of this solution add 400 mL of acetonitrile and 100 mL of tetrahydrofuran.
Flow rate: Adjust so that the retention time of diflorasone diacetate is about 15 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and diflorasone diacetate are eluted in this order with the resolution between these peaks being not less than 9.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diflorasone diacetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Diflucortolone Valerate

ジフルコルトロン吉草酸エステル



$C_{27}H_{36}F_2O_5$: 478.57
6α,9-Difluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pentanate
[*59198-70-8*]

Diflucortolone Valerate contains not less than 98.0% and not more than 102.0% of diflucortolone valerate ($C_{27}H_{36}F_2O_5$), calculated on the dried basis.

Description Diflucortolone Valerate occurs as white, crystals or crystalline powder.
It is sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Prepare the test solution by proceeding with 10 mg of Diflucortolone Valerate according to the Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ for fluoride.
(2) Determine the absorption spectrum of a solution of Diflucortolone Valerate in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Diflucortolone Valerate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(3) Determine the infrared absorption spectrum of Diflucortolone Valerate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Diflucortolone Valerate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +110 – +115° (0.1 g calculated on the dried basis, ethanol (99.5), 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 200 – 204°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Diflucortolone Valerate in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm). Carbonize and incinerate as directed under Residue on Ignition ⟨*2.44*⟩.
(2) Related substances—Use the sample solution obtained in the Assay as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of sample solution by the

automatic integration method, and calculate the amounts of these peaks by the area percentage method: the amount of each peak of flucortolone valerate, 12α diflucortolone valerate and Δ4 diflucortolone valerate, having the relative retention times of about 0.97, 1.03 and 1.05 to diflucortolone valerate, respectively, is not more than 0.6%, respectively; the amount of the peak of clocortolone valerate, having the relative retention time of about 1.09, is not more than 0.3%; and the amount of each peak other than those mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than diflucortolone valerate is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 1.4 times as long as the retention time of diflucortolone valerate, beginning after the solvent peak.

System suitability—

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To 0.1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add a mixture of water and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of diflucortolone valerate obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of diflucortolone valerate obtained from 10 μL of the solution for system suitability test.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 5 mg each of Diflucortolone Valerate and Diflucortolone Valerate RS (separately, determine the loss on drying ⟨*2.41*⟩ under the same conditions as Diflucortolone Valerate), dissolve each in a mixture of water and acetonitrile (1:1) to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of diflucortolone valerate in each solution.

Amount (mg) of diflucortolone valerate ($C_{27}H_{36}F_2O_5$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Diflucortolone Valerate RS taken, calculated on dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with sulfonamide group bound to hexadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, adjusted to pH 3.0 with phosphoric acid, and acetonitrile for liquid chromatography (11:9).

Mobile phase B: Acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	100 → 90	0 → 10
10 – 25	90	10
25 – 45	90 → 35	10 → 65
45 – 50	35	65

Flow rate: 1.0 mL per minute.

System suitability—

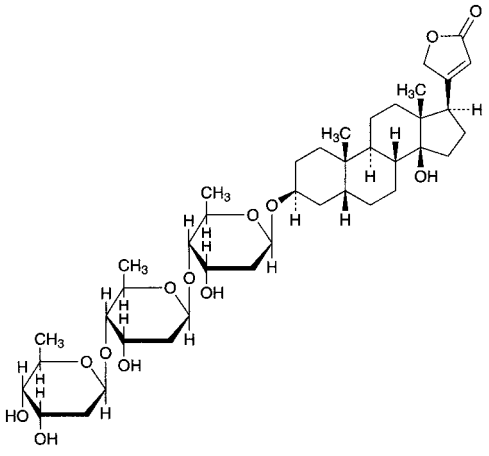
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of diflucortolone valerate are not less than 10,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diflucortolone valerate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Digitoxin

ジギトキシン



$C_{41}H_{64}O_{13}$: 764.94

3β-[2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-14-hydroxy-5β,14β-card-20(22)-enolide

[*71-63-6*]

Digitoxin, when dried, contains not less than 90.0% of digitoxin ($C_{41}H_{64}O_{13}$).

Description Digitoxin occurs as a white to light yellowish white, crystalline powder. It is odorless.

It is soluble in chloroform, sparingly soluble in methanol and in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification **(1)** Transfer 1 mg of Digitoxin to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids a brown ring free from a reddish color is produced, and the color of the

upper layer near the contact zone changes to green through purple. Finally the color of the entire acetic acid layer changes to green through deep blue.

(2) To 2 mg of Digitoxin add 25 mL of a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100), and dissolve by shaking. Take 2 mL of this solution, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and mix: a red-purple color develops slowly, and then fades.

(3) Dissolve 1 mg each of Digitoxin and Digitoxin RS in a mixture of chloroform and ethanol (95) (1:1) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat at 110°C for 10 minutes: the spot from the sample solution shows the same *R*f value as the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_D^{20}$: +16 – +18° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity Digitonin—Dissolve 10 mg of Digitoxin in 2 mL of ethanol (95) in a test tube, having the inner walls which are free from scratches, add 2 mL of a solution of cholesterol in ethanol (95) (1 in 200), mix gently, and allow to stand for 10 minutes: no turbidity is produced.

Loss on drying <2.41> Not more than 1.5% (0.5 g, in vacuum, 100°C, 2 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Dissolve about 20 mg each of Digitoxin and Digitoxin RS, previously dried and accurately weighed, in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution to each solution, add 12.5 mL of water, then add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of digitoxin to that of the internal standard.

$$\text{Amount (mg) of digitoxin } (C_{41}H_{64}O_{13}) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Digitoxin RS taken

Internal standard solution—A solution of acenaphthene in methanol (3 in 1,000,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol and water (3:1).

Flow rate: Adjust so that the retention time of digitoxin is about 5 minutes.

Selection of column: Proceed with 50 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of digitoxin and the internal standard in this order with the resolution between these peaks being not less than 6.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Digitoxin Tablets

ジギトキシン錠

Digitoxin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of digitoxin ($C_{41}H_{64}O_{13}$: 764.94).

Method of preparation Prepare as directed under Tablets, with Digitoxin.

Identification **(1)** Place a portion of powdered Digitoxin Tablets, equivalent to 2 mg of digitoxin ($C_{41}H_{64}O_{13}$), in a separator, shake with 30 mL of water, and shake vigorously with 30 mL of chloroform. Filter the chloroform extract with a funnel on which a small amount of anhydrous sodium sulfate is placed, and transfer to a round-bottomed flask connected by a universal joint. Evaporate the solution to dryness by warming under reduced pressure, and dissolve the residue in 10 mL of chloroform. Transfer 5 mL of this solution to a small test tube about 10 mm in inside diameter, and evaporate to dryness on a water bath with the aid of a current of air. Proceed with the residue as directed in the Identification (1) under Digitoxin.

(2) Evaporate 4 mL of the chloroform solution obtained in (1) to dryness, by warming under reduced pressure, add a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100) to the residue, and dissolve by shaking. Proceed with 2 mL of this solution as directed in the Identification (2) under Digitoxin.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Digitoxin Tablets to a 50-mL beaker, add 0.5 mL of water to disintegrate the tablet, add 5 mL of acetonitrile, and warm on a water bath for 5 minutes, covering the beaker with a watch glass. After cooling, transfer the solution to separator A, rinse the beaker with 30 mL of chloroform and then with 20 mL of water, transfer the rinsings to separator A, and extract by vigorous shaking. Transfer the chloroform extract to separator B containing 5 mL of a solution of sodium hydrogen carbonate (1 in 100), and shake to wash. Filter the chloroform layer through a pledget of absorbent cotton, previously moistened with chloroform. Extract the water layer in separator A with two 30-mL portions of chloroform, wash the chloroform extract with a solution of sodium hydrogen carbonate (1 in 100) in separator B, filter in the same manner, and combine the filtrate with the first one. Evaporate this filtrate to dryness under reduced pressure by warming, add diluted ethanol (95) (4 in 5) to make exactly *V* mL of a solution containing 5 μg of digitoxin ($C_{41}H_{64}O_{13}$) per ml. Shake vigorously for 20 minutes to dissolve, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Digitoxin RS, previously dried at 100°C for 2 hours, and dissolve in diluted ethanol (95) (4 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted ethanol (95) (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and diluted ethanol (95) (4 in 5) into brown glass-stoppered test tubes T, S and B. Add exactly 10 mL each of

0.02 w/v% L-ascorbic acid-hydrochloric acid TS, shake well, and immediately add exactly 1 mL each of dilute hydrogen peroxide TS. Shake vigorously, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F_T, F_S and F_B, of these solutions at 400 nm of the excitation wavelength and at about 570 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

$$\text{Amount (mg) of digitoxin } (C_{41}H_{64}O_{13}) = M_S \times (F_T - F_B)/(F_S - F_B) \times V/2000$$

M_S: Amount (mg) of Digitoxin RS taken

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Basket method, using 500 mL of diluted hydrochloric acid (3 in 500) as the dissolution medium, the dissolution rates in 30 minutes and in 60 minutes of Digitoxin Tablets are not less than 60% and 85%, respectively. No retest requirement is applied to Digitoxin Tablets.

Start the test with 1 tablet of Digitoxin Tablets, withdraw α + 15 mL of the medium at the specified minute after starting the test, immediately add the same volume of fresh dissolution medium, previously warmed at 37 ± 0.5°C, to the vessel, and filter withdrawing medium through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Measure exactly a mL of the sample solution, equivalent to about 2 μg of digitoxin ($C_{41}H_{64}O_{13}$), transfer to a glass-stoppered centrifuge tube T_{30}, and warm at 37 ± 0.5°C for 30 minutes. Further, at 60 minutes after starting the test, take a + 15 mL of the dissolved solution, proceed in the same manner as above, measure exactly a mL of the sample solution so obtained, and transfer to a glass-stoppered centrifuge tube T_{60}. Separately, weigh accurately 100 times the labeled amount of Digitoxin RS, previously dried under reduced pressure at 100°C for 2 hours, and dissolve in ethanol (95) to make exactly 100 mL. Measure exactly 1 mL of this solution, add the dissolution medium to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and filter through a membrane filter (less than 0.8 μm in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the standard solution. Measure exactly a mL each of the standard solution and the dissolution medium, transfer to glass-stoppered centrifuge tubes T_S and T_B, respectively. Add exactly 7 mL of chloroform to each of the glass-stoppered centrifuge tubes T_{30}, T_{60}, T_S and T_B, shake vigorously for 10 minutes and centrifuge. Discard the aqueous layer, measure exactly 5 mL of the chloroform layer, transfer to brown test tubes T'_{30}, T'_{60}, T'_S and T'_B, evaporate the chloroform, add exactly 4 mL each of 0.05 g/dL L-ascorbic acid-hydrochloric acid TS, shake well, and allow to stand for 10 minutes. Then add exactly 0.5 mL each of dilute hydrogen peroxide TS, shake well, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F_{30}, F_{60}, F_S and F_B, of these solutions at about 395 nm of the excitation wavelength and at about 560 nm of the fluorescence wavelength as directed under Fluorometry <2.22>.

$$\text{Dissolution rate (\%) with respect to the labeled amount of digitoxin } (C_{41}H_{64}O_{13}) \text{ for 30 minutes} = M_S \times (F_{30} - F_B)/(F_S - F_B) \times 1/C$$

$$\text{Dissolution rate (\%) with respect to the labeled amount of digitoxin } (C_{41}H_{64}O_{13}) \text{ for 60 minutes} = M_S \times \left(\frac{F_{60} - F_B}{F_S - F_B} + \frac{F_{30} - F_B}{F_S - F_B} \times \frac{a + 15}{500}\right) \times 1/C$$

M_S: Amount (mg) of Digitoxin RS taken

C: The labeled amount (mg) of digitoxin ($C_{14}H_{64}O_{13}$) in 1 tablet

a + 15: Volume (mL) of dissolved solution taken at the specified minute

Assay Weigh accurately and powder not less than 20 Digitoxin Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of digitoxin ($C_{41}H_{64}O_{13}$), and shake with 12.5 mL of water for 10 minutes. Add exactly 10 mL of the internal standard solution, shake for 20 minutes, and add methanol to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Digitoxin RS, previously dried in vacuum at 100°C for 2 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of the solution, add exactly 10 mL of the internal standard solution, add 12.5 mL of water, then methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Digitoxin.

$$\text{Amount (mg) of digitoxin } (C_{41}H_{64}O_{13}) = M_S \times Q_T/Q_S \times 1/40$$

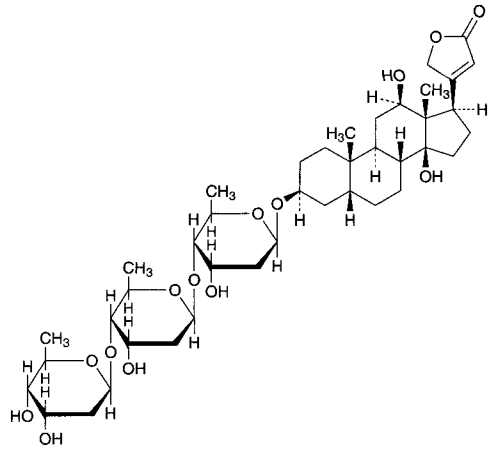
M_S: Amount (mg) of Digitoxin RS taken

Internal standard solution—A solution of acenaphthene in methanol (3 in 1,000,000).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Digoxin

ジゴキシン



$C_{41}H_{64}O_{14}$: 780.94
3β-[2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide
[*20830-75-5*]

Digoxin, when dried, contains not less than 96.0% and not more than 106.0% of digoxin ($C_{41}H_{64}O_{14}$).

Description Digoxin occurs as colorless or white crystals or a white crystalline powder.

It is freely soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in acetic acid (100), and practically insoluble in water.

Identification **(1)** Transfer 1 mg of Digoxin to a small test

tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids a brown ring free from a reddish color is produced, and the color of the upper layer near the contact zone changes to green through purple. Finally the entire acetic acid layer shows a green color through a deep blue color.

(2) Determine the infrared absorption spectrum of Digoxin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +10.0 – +13.0° (after drying, 0.20 g, dehydratead pyridine, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Digoxin in 15 mL of diluted ethanol (95) (4 in 5) by warming at 70°C: the solution is clear and colorless.

(2) Related substances—Dissolve 25.0 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water and dilute ethanol to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 5.0 mg of Gitoxin RS, previously dried under reduced pressure at 105°C for 1 hour, in a mixture of acetonitrile and water (7:3) to make exactly 200 mL. Pipet 2 mL of this solution, add dilute ethanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of gitoxin: A_T is not larger than A_S, and the total of the areas of the peaks other than digitoxin and gitoxin, obtained by the area percentage method, is not more than 3%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of digoxin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL of this solution is equivalent to 0.07 to 0.13% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

Loss on drying <2.41> Not more than 1.0% (0.5 g, in vacuum, 105°C, 1 hour).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Weigh accurately about 25 mg each of Digoxin and Digoxin RS, previously dried, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of these solutions, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of digoxin to that of the internal standard.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Digoxin RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust so that the retention time of digoxin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Digoxin Injection

ジゴキシン注射液

Digoxin Injection is an aqueous injection.

It contains not less than 90.0% and not more than 105.0% of the labeled amount of digoxin ($C_{41}H_{64}O_{14}$: 780.94).

Method of preparation Prepare as directed under Injections, with a solution of Digoxin in 10 to 50 vol% ethanol.

Description Digoxin Injection is a clear, colorless liquid.

Identification Dilute Digoxin Injection, if necessary, with methanol so that each mL contains about 0.25 mg of Digoxin, and use this solution as the sample solution. In case where ingredients are suspected to affect the test, remove

them by means of a solid-phase extraction. Separately, dissolve 0.5 mg of Digoxin RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of a solution of trichloroacetic acid in ethanol (99.5) (1 in 4) and a freshly prepared solution of sodium toluenesulfonchloramide trihydrate (3 in 100) (4:1) on the plate, heat at 110°C for 10 minutes, and examine under ultraviolet light (main wavelength: 366 nm): the *R*f values of the principal spots with the sample solution and the standard solution are not different each other.

Alcohol number ⟨*1.01*⟩ 0.8 – 1.2 (Method 1).

Purity Related substances—To a volume of Digoxin Injection, equivalent to about 2.5 mg of Digoxin, add dilute ethanol to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than digoxin is not more than 5%.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of digoxin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL this solution is equivalent to 0.07 to 0.13% of that of digoxin obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

Bacterial endotoxins ⟨*4.01*⟩ Less than 200 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirements.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ Perform the test according to Method 1: it meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Digoxin Injection, equivalent to about 2.5 mg of digoxin ($C_{41}H_{64}O_{14}$), add exactly 5 mL of the internal standard solution and dilute ethanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of digoxin to that of the internal standard.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of Digoxin RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust so that the retention time of digoxin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Digoxin Tablets

ジゴキシン錠

Digoxin Tablets contain not less than 90.0% and not more than 105.0% of the labeled amount of digoxin ($C_{41}H_{64}O_{14}$: 780.94).

Method of preparation Prepare as directed under Tablets, with Digoxin.

Identification To an amount of powdered Digoxin Tablets, equivalent to 0.5 mg of Digoxin, add 2 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 0.5 mg of Digoxin RS in 2 mL of methanol, and use this solution as the standard solution.

Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of a solution of trichloroacetic acid in ethanol (99.5) (1 in 4) and a freshly prepared solution of sodium toluenesulfonchloramide trihydrate (3 in 100) (4:1) on the plate, heat at 110°C for 10 minutes, and examine under ultraviolet light (main wavelength: 366 nm): the *R*f values of the principal spots with the sample solution and the standard solution are not different each other.

Purity Related substances—Powder not less than 20 Digoxin Tablets. Weigh a portion of the powder equivalent to 2.5 mg of Digoxin, add 30 mL of dilute ethanol, treat with ultrasonic waves for 20 minutes, and shake for 5 minutes. After cooling, add dilute ethanol to make 50 mL, filter, and use the filtrate as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amount of these peaks by the area percentage method: the total amount of the peaks other than digoxin is not more than 5%.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of digoxin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL of this solution is equivalent to 0.07 to 0.13% of that of digoxin obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Digoxin Tablets add 0.5 mL of water to disintegrate, then add exactly 0.5 mL of the internal standard solution, and add *V* mL of dilute ethanol so that each mL contains about 21 μg of digoxin ($C_{41}H_{64}O_{14}$). Exposure this solution to ultrasonic waves for 20 minutes, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, and add ethanol (95) to make exactly 20 mL. Pipet 1 mL of this solution, add exactly 0.5 mL of the internal standard solution, then add 1.5 mL of water and (V – 2) mL of dilute ethanol, and use this solution as the standard solution. Proceed with the sample solution and standard solution as directed in the Assay.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = M_S \times Q_T/Q_S \times 1/200$$

M_S: Amount (mg) of Digoxin RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 40,000/V).

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Basket method, using 500 mL of diluted hydrochloric acid (3 in 500) as the dissolution medium, the dissolution rate in 60 minutes of Digoxin Tablets is not less than 65%. No retest requirement is applied to Digoxin Tablets.

Start the test with 1 tablet of Digoxin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried in vacuum at 105°C for 1 hour, dissolve in a small portion of ethanol (95), and add a mixture of ethanol (95) and water (4:1) to make exactly 500 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 500 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and the dissolution medium, and transfer to brown glass-stoppered test tubes. Add exactly 10 mL of 0.012 g/dL L-ascorbic acid-hydrochloric acid TS to these tubes, and shake. Immediately add exactly 1 mL of dilute hydrogen peroxide TS, shake well, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F_T, F_S, and F_B, of these solutions at 360 nm of the excitation wavelength and at 485 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

$$\text{Dissolution rate (\%) with respect to the labeled amount of digoxin } (C_{41}H_{64}O_{14}) = M_S \times (F_T - F_B)/(F_S - F_B) \times 1/C$$

M_S: Amount (mg) of Digoxin RS taken

C: The labeled amount (mg) of digoxin ($C_{41}H_{64}O_{14}$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Digoxin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2.5 mg of digoxin ($C_{41}H_{64}O_{14}$), add 30 mL of dilute ethanol, exposure to ultrasonic waves for 20 minutes, and shake for 5 minutes. Add exactly 5 mL of the internal standard solution and dilute ethanol to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Digoxin RS, previously dried under reduced pressure at 105°C for 1 hour , dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard

solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of digoxin to that of the internal standard.

$$\text{Amount (mg) of digoxin } (C_{41}H_{64}O_{14}) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of Digoxin RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust so that the retention time of digoxin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

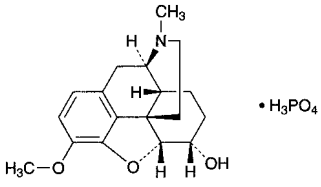
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dihydrocodeine Phosphate

ジヒドロコデインリン酸塩



$C_{18}N_{23}NO_3.H_3PO_4$: 399.38

(5*R*,6*S*)-4,5-Epoxy-3-methoxy-17-methylmorphinan-6-ol monophosphate

[*24204-13-5*]

Dihydrocodeine Phosphate contains not less than 98.0% of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$), calculated on the dried basis.

Description Dihydrocodeine Phosphate occurs as a white to yellowish white crystalline powder.

It is freely soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Dihydrocodeine Phosphate in 10 mL of water is between 3.0 and 5.0.

It is affected by light.

Identification **(1)** Determine the absorption spectrum of a solution of Dihydrocodeine Phosphate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Dihydrocodeine Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dihydrocodeine Phosphate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for phosphate.

Purity **(1)** Chloride <*1.03*>—Perform the test with 0.5 g of Dihydrocodeine Phosphate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) Sulfate <*1.14*>—Perform the test with 0.20 g of Dihydrocodeine Phosphate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Related substances—Dissolve 0.20 g of Dihydrocodeine Phosphate in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g of Dihydrocodeine Phosphate, dissolve in 70 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 39.94 \text{ mg of } C_{18}H_{23}NO_3.H_3PO_4$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

1% Dihydrocodeine Phosphate Powder

ジヒドロコデインリン酸塩散 1%

1% Dihydrocodeine Phosphate Powder contains not less than 0.90% and not more than 1.10% of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$: 399.38).

Method of preparation

Dihydrocodeine Phosphate	10 g
Lactose Hydrate	a sufficient quantity
To make	1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 1% Dihydrocodeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 281 nm and 285 nm.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of 1% Dihydrocodeine Phosphate Powder is not less than 85%.

Start the test with about 1 g of 1% Dihydrocodeine Phosphate Powder, accurately weighed, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay (separately determine the loss on drying <2.41> at 105°C for 4 hours), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of dihydrocodeine in each solution.

Dissolution rate (%) with respect to the labeled amount of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$)
$= M_S/M_T \times A_T/A_S \times 9/5$

M_S: Amount (mg) of dihydrocodeine phosphate for assay taken, calculated on the dried basis
M_T: Amount (g) of 1% Dihydrocodeine Phosphate Powder taken

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dihydrocodeine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dihydrocodeine is not more than 2.0%.

Assay Weigh accurately about 5 g of 1% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay (separately determine the loss on drying <2.41> at 105°C for 4 hours), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dihydrocodeine to that of the internal standard.

Amount (mg) of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of dihydrocodeine phosphate for assay taken, calculated on the dried basis

Internal standard solution—A solution of ethylefurin hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.

Flow rate: Adjust so that the retention time of dihydrocodeine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

10% Dihydrocodeine Phosphate Powder

ジヒドロコデインリン酸塩散 10%

10% Dihydrocodeine Phosphate Powder contains not less than 9.3% and not more than 10.7% of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$: 399.38).

Method of preparation

Dihydrocodeine Phosphate	100 g
Lactose Hydrate	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10% Dihydrocodeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 281 nm and 285 nm.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of 10% Dihydrocodeine Phosphate Powder is not less than 85%.

Start the test with about 0.1 g of 10% Dihydrocodeine Phosphate Powder, accurately weighed, withdraw not less

than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of dihydrocodeine phosphate for assay (separately determine the loss on drying <2.41> at 105°C for 4 hours), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of dihydrocodeine in each solution.

Dissolution rate (%) with respect to the labeled amount of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$)
$= M_S/M_T \times A_T/A_S \times 9/20$

M_S: Amount (mg) of dihydrocodeine phosphate for assay taken, calculated on the dried basis
M_T: Amount (g) of 10% Dihydrocodeine Phosphate Powder taken

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dihydrocodeine are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dihydrocodeine is not more than 2.0%.

Assay Weigh accurately about 2.5 g of 10% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay, (separately determine the loss on drying <2.41> at 105°C for 4 hours), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dihydrocodeine to that of the internal standard.

Amount (mg) of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$)
$= M_S \times Q_T/Q_S \times 5$

M_S: Amount (mg) of dihydrocodeine phosphate for assay taken, calculated on the dried basis

Internal standard solution—A solution of ethylefrine hydrochloride (3 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.
Flow rate: Adjust so that the retention time of dihydrocodeine is about 9 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Dihydroergotamine Mesilate

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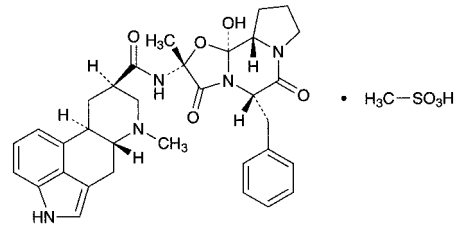

$C_{33}H_{37}N_5O_5.CH_4O_3S$: 679.78
(5′*S*,10*R*)-5′-Benzyl-12′-hydroxy-2′-methyl-9,10-dihydroergotaman-3′,6′,18-trione monomethanesulfonate
[*6190-39-2*]

Dihydroergotamine Mesilate contains not less than 97.0% of dihydroergotamine mesilate ($C_{33}H_{37}N_5O_5.CH_4O_3S$), calculated on the dried basis.

Description Dihydroergotamine Mesilate occurs as a white to yellowish white or grayish white to reddish white powder.
It is freely soluble in acetic acid (100), sparingly soluble in methanol and in chloroform, slightly soluble in water and in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.
It is gradually colored by light.
Melting point: about 214°C (with decomposition).

Identification **(1)** Dissolve 1 mg of Dihydroergotamine Mesilate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, and shake: a blue color develops.
(2) To 0.1 g of Dihydroergotamine Mesilate add 0.4 g of sodium hydroxide, stir well, and incinerate by gradual ignition. After cooling, add 10 mL of water to the residue, heat to boiling, cool, and filter. To the filtrate add 0.5 mL of hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for sulfate. Separately, to 0.1 g of Dihydroergotamine Mesilate add 5 mL of dilute hydrochloric acid, shake for 5 minutes, filter, and to the filtrate add 1 mL of barium chloride TS: the solution is clear.
(3) Determine the absorption spectrum of a solution of

Dihydroergotamine Mesilate in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Dihydroergotamine Mesilate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: −16.7 – −22.7° [0.5 g, calculated on the dried basis, a mixture of ethanol (99.5), chloroform and ammonia solution (28) (10:10:1), 20 mL, 100 mm].

pH <2.54> Dissolve 0.05 g of Dihydroergotamine Mesilate in 50 mL of water: the pH of this solution is between 4.4 and 5.4.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Dihydroergotamine Mesilate in 0.1 mL of a solution of methanesulfonic acid (7 in 100) and 50 mL of water: the solution is clear, and has no more color than the following control solutions [1] or [2].

Control solution [1]: Pipet 0.6 mL of Iron (III) Chloride CS and 0.15 mL of Cobalt (II) Chloride CS, mix, and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution [2]: Pipet 0.6 mL of Iron (III) Chloride CS, 0.25 mL of Cobalt (II) Chloride CS and 0.1 mL of Copper (II) Sulfate CS, mix, and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Dihydroergotamine Mesilate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add a mixture of chloroform and methanol (9:1) to make exactly 25 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:6:1) to a distance of about 15 cm, and dry the plate with cold wind within 1 minute. Develop the plate again immediately with a freshly prepared mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:6:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and dry the plate with warm wind: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the spots, which are more intense than the spot from the standard solution (2), are not more than two.

Loss on drying <2.41> Not more than 4.0% (0.5 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 6 hours).

Assay Weigh accurately about 0.2 g of Dihydroergotamine Mesilate, dissolve in 170 mL of a mixture of acetic anhydride and acetic acid (100) (10:1), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 13.60 mg of $C_{33}H_{37}N_5O_5.CH_4O_3S$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Dihydroergotoxine Mesilate

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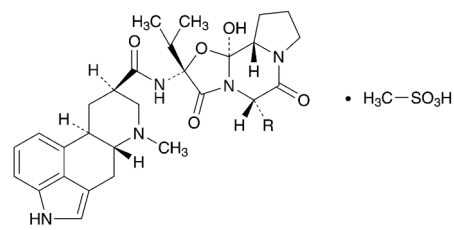

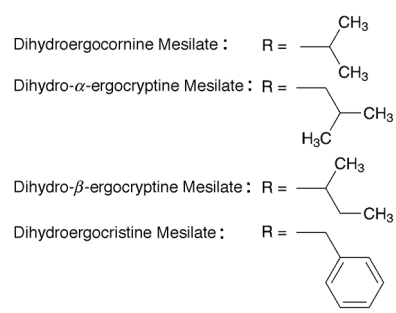

Dihydroergocornine Mesilate
$C_{31}H_{41}N_5O_5.CH_4O_3S$: 659.79
(5′*S*,10*R*)-12′-Hydroxy-2′,5′-bis(1-methylethyl)-9,10-dihydroergotaman-3′,6′,18-trione monomethanesulfonate

Dihydro-α-ergocryptine Mesilate
$C_{32}H_{43}N_5O_5.CH_4O_3S$: 673.82
(5′*S*,10*R*)-12′-Hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)-9,10-dihydroergotaman-3′,6′,18-trione monomethanesulfonate

Dihydro-β-ergocryptine Mesilate
$C_{32}H_{43}N_5O_5.CH_4O_3S$: 673.82
(5′*S*,10*R*)-12′-Hydroxy-2′-(1-methylethyl)-5′-(1-methylpropyl)-9,10-dihydroergotaman-3′,6′,18-trione monomethanesulfonate

Dihydroergocristine Mesilate
$C_{35}H_{41}N_5O_5.CH_4O_3S$: 707.84
(5′*S*,10*R*)-5′-Benzyl-12′-hydroxy-2′-(1-methylethyl)-9,10-dihydroergotaman-3′,6′,18-trione monomethanesulfonate

[*8067-24-1*, Dihydroergotoxine Mesilate]

Dihydroergotoxine Mesilate contains not less than 97.0% and not more than 103.0% of dihydroergotoxine mesilate [as a mixture of dihydroergocornine mesilate ($C_{31}H_{41}N_5O_5.CH_4O_3S$), dihydro-α-ergocryptine mesilate ($C_{32}H_{43}N_5O_5.CH_4O_3S$), dihydro-β-ergocryptine mesilate ($C_{32}H_{43}N_5O_5.CH_4O_3S$) and dihydroergocristine mesilate ($C_{35}H_{41}N_5O_5.CH_4O_3S$)], calculated on the anhydrous basis. The relative contents of dihydroergocornine mesilate ($C_{31}H_{41}N_5O_5.CH_4O_3S$), dihydroergocryptine mesilate ($C_{32}H_{43}N_5O_5.CH_4O_3S$) and dihydroergocristine mesilate ($C_{35}H_{41}N_5O_5.CH_4O_3S$) are 30.3–36.3% each, and the content ratio of dihydro-α-ergocryptine mesilate and dihydro-β-

ergocryptine mesilate is 1.5–2.5:1.

Description Dihydroergotoxine Mesilate occurs as a white to pale yellow powder.

It is soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, in acetonitrile and in chloroform, and practically insoluble in diethyl ether.

Identification Determine the infrared absorption spectrum of Dihydroergotoxine Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +11.0 – +15.0° (0.2 g calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Dihydroergotoxine Mesilate in 20 mL of water: the solution is clear and the color of the solution is not more intense than that of the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 0.4 mL of Copper (II) Sulfate CS and 2.4 mL of Iron (III) Chloride CS add diluted hydrochloric acid (1 in 40) to make exactly 200 mL.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Dihydroergotoxine Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately 0.100 g of Dihydroergotoxine Mesilate, dissolve it in a mixture of chloroform and methanol (9:1) to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately 10 mg of dihydroergocristine mesilate for thin-layer chromatography, and dissolve in a mixture of chloroform and methanol (9:1) to make exactly 100 mL. Pipet 6 mL, 4 mL and 2 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 10 mL, respectively, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03> without putting the filter paper in the developing vessel. Spot 5 μL each of the sample solution and the standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate with the aid of a cool air stream. Immediately after that, develop the plate again with a newly prepared mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate within 1 minute with the aid of a cool air stream. Spray evenly *p*-dimethylaminobenzal-dehyde-hydrochloric acid TS on the plate, dry the plate within 2 minutes with the aid of a cool air stream, and heat it at 40°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), not more than 2 spots are more intense than that from the standard solution (2), and not more than 4 spots are more intense than that from the standard solution (3).

Water <2.48> Not more than 5.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay **(1)** Dihydroergotoxine mesilate—Weigh accurately about 30 mg each of Dihydroergotoxine Mesilate and Dihydroergotoxine Mesilate RS, and dissolve them separately in a suitable amount of a mixture of water and acetonitrile (3:1). To these solutions add exactly 10 mL of the internal standard solution and an amount of a mixture of water and acetonitrile (3:1) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios of the peak areas of dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine to the peak area of the internal standard of these solutions.

$$\text{Amount (mg) of dihydroergotoxine mesilate} = M_S \times (Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD})/(Q_{SA} + Q_{SB} + Q_{SC} + Q_{SD})$$

M_S: Amount (mg) of Dihydroergotoxine Mesilate RS taken, calculated on the anhydrous basis

Q_{TA}: Ratio of the peak area of dihydroergocornine to that of the internal standard of the sample solution × 659.80

Q_{TB}: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the sample × 673.83

Q_{TC}: Ratio of the peak area of dihydroergocristine to that of the internal standard of the sample solution × 707.85

Q_{TD}: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the sample solution × 673.83

Q_{SA}: Ratio of the peak area of dihydroergocornine to that of the internal standard of the standard solution × 659.80

Q_{SB}: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the standard solution × 673.83

Q_{SC}: Ratio of the peak area of dihydroergocristine to that of the internal standard of the standard solution × 707.85

Q_{SD}: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the standard solution × 673.83

Internal standard solution—Dissolve 0.04 g of chloramphenicol in a mixture of water and acetonitrile (3:1) to make 250 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and triethylamine (30:10:1).

Flow rate: Adjust so that the retention time of chloramphenicol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard, dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine are eluted in this order with the resolution between the peaks of dihydro-α-ergocryptine and dihydroergocristine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the ratios of the peak area of dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine to that of the internal standard is not more than 0.5%.

(2) Relative contents of dihydroergocornine mesilate, dihydroergocryptine mesilate and dihydroergocristine mesilate—Calculate the relative amounts of dihydroergocornine mesilate, dihydroergocryptine mesilate (dihydro-α-ergocryptine mesilate and dihydro-β-ergocryptine mesilate) and dihydroergocristine mesilate from the chromatogram obtained in Assay (1) for the sample solution using the following equations:

Relative amount (%) of dihydroergocornine mesilate
$= Q_{TA}/(Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD}) \times 100$

Relative amount (%) of dihydroergocryptine mesilate
$= (Q_{TB} + Q_{TD})/(Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD}) \times 100$

Relative amount (%) of dihydroergocristine mesilate
$= Q_{TC}/(Q_{TA} + Q_{TB} + Q_{TC} + Q_{TD}) \times 100$

(3) Ratio of the content of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate—Calculate the ratio of the amount of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate from the chromatogram obtained in the Assay (1) for the sample solution using the following equations:

Ratio of the content of dihydro-α-ergocryptine mesilate to dihydro-β-ergocryptine mesilate
$= Q_{TB}/Q_{TD}$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dilazep Hydrochloride Hydrate

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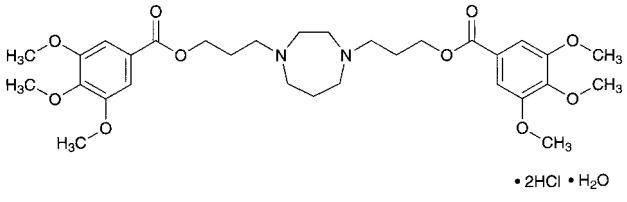

$C_{31}H_{44}N_2O_{10}.2HCl.H_2O$: 695.63
3,3′-(1,4-Diazepane-1,4-diyl)dipropyl bis(3,4,5-trimethoxybenzoate) dihydrochloride monohydrate
[*20153-98-4*, anhydride]

Dilazep Hydrochloride Hydrate contains not less than 98.0% of dilazep hydrochloride ($C_{31}H_{44}N_2O_{10}.2HCl$: 677.62), calculated on the dried basis.

Description Dilazep Hydrochloride Hydrate occurs as a white crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in water, slightly soluble in ethanol (95) and in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: 200 – 204°C Immerse the sample in a bath of 110°C, and raise the temperature at the rate of about 3°C per minute from 140°C to 150°C, about 10°C per minute from 160°C to 195°C and about 1°C per minute from 195°C.

Identification (1) To 1 mL of a solution of Dilazep Hydrochloride Hydrate (1 in 100) add 0.1 mL of a solution of hydroxylammonium chloride (1 in 10) and 0.1 mL of 8 mol/L potassium hydroxide TS, and warm in a water bath of 70°C for 10 minutes. After cooling, add 0.5 mL of dilute hydrochloric acid and 0.1 mL of iron (III) chloride TS: a purple color develops.

(2) To 5 mL of a solution of Dilazep Hydrochloride Hydrate (3 in 500) add 0.3 mL of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Dilazep Hydrochloride Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Dilazep Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Perform the test with 0.5 g of Dilazep Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Dilazep Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Dilazep Hydrochloride Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.40 g of Dilazep Hydrochloride Hydrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate, dichloromethane and hydrochloric acid (500:200:100:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> 2.0 – 3.0% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Dilazep Hydrochloride Hydrate, dissolve in 40 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.88 mg of $C_{31}H_{44}N_2O_{10}.2HCl$

Containers and storage Containers—Tight containers.

Diltiazem Hydrochloride

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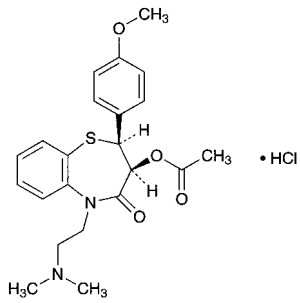


$C_{22}H_{26}N_2O_4S.HCl$: 450.98
(2*S*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate monohydrochloride
[*33286-22-5*]

Diltiazem Hydrochloride, when dried, contains not less than 98.5% of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S.HCl$).

Description Diltiazem Hydrochloride occurs as white, crystals or crystalline powder. It is odorless.

It is very soluble in formic acid, freely soluble in water, in methanol and in chloroform, sparingly soluble in acetonitrile, slightly soluble in acetic anhydride and in ethanol (99.5), and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 0.05 g of Diltiazem Hydrochloride in 1 mL of 1 mol/L hydrochloric acid TS, add 2 mL of ammonium thiocyanate-cobalt (II) nitrate TS and 5 mL of chloroform, shake well, and allow to stand: a blue color develops in the chloroform layer.

(2) Proceed as directed under Oxygen Flask Combustion Method <*1.06*> with 0.03 g of Diltiazem Hydrochloride, using 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution responds to the Qualitative Tests <*1.09*> (1) for sulfate.

(3) Dissolve 0.01 g of Diltiazem Hydrchloride in 0.01 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of the solution add 0.01 mol/L hydrochloric acid TS to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Diltiazem Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1741 cm^{-1}, 1678 cm^{-1}, 1252 cm^{-1} and 1025 cm^{-1}.

(5) A solution of Diltiazem Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +115 – +120° (after drying, 0.20 g, water, 20 mL, 100 mm).

Melting point <*2.60*> 210 – 215°C (with decomposition).

pH <*2.54*> Dissolve 1.0 g of Diltiazem Hydrochloride in 100 mL of water: the pH of this solution is between 4.3 and 5.3.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Diltiazem Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Perform the test with 1.0 g of Diltiazem Hydrochloride. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Diltiazem Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Place 1.0 g of Diltiazem Hydrochloride in a decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the neck of the flask, and heat cautiously until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution becomes colorless to pale yellow. After cooling, add 2 mL of saturated solution of ammonium oxalate monohydrate, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, use this solution as the test solution, and perform the test: the test solution has no more color than the following control solution (not more than 2 ppm).

Control solution: Proceed in the same manner as the test solution without Diltiazem Hydrochloride, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and proceed in the same manner as the test solution.

(5) Related substances—Dissolve 50 mg of Diltiazem Hydrochloride in 50 mL of diluted ethanol (4 in 5), and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add diluted ethanol (4 in 5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of peaks other than the peak of diltiazem obtained from the sample solution is not larger than 3/5 times the peak area of diltiazem obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8 g of sodium acetate trihydrate and 1.5 g of *d*-camphorsulfonic acid in 500 mL of water, and filter using a membrane filter (0.4 μm in pore size). Add 250 mL each of acetonitrile and methanol to the filtrate.

Flow rate: Adjust so that the retention time of diltiazem is about 9 minutes.

Time span of measurement: About twice as long as the retention time of diltiazem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add diluted ethanol (4 in 5) to make exactly 10 mL. Confirm that the peak area of diltiazem obtained from 20 μL of this solution is equivalent to 15 to 25% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 0.03 g of Diltiazem Hydrochloride, 0.02 g of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4-(5*H*)-one hydrochloride (hereinafter referred to as

de-acetyl substance) and 0.02 g of phenylbenzoate in 160 mL of ethanol (99.5), and add water to make 200 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, de-acetyl substance, diltiazem and phenyl benzoate are eluted in this order with the resolutions between the peaks of de-acetyl substance and diltiazem and between the peaks of diltiazem and phenyl benzoate being not less than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diltiazem is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Diltiazem Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 45.10 mg of $C_{22}H_{26}N_2O_4S.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Diltiazem Hydrochloride Extended-release Capsules

ジルチアゼム塩酸塩徐放カプセル

Diltiazem Hydrochloride Extended-release Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S.HCl$: 450.98).

Method of preparation Prepare as directed under Capsules, with Diltiazem Hydrochloride.

Identification Take out the content of Diltiazem Hydrochloride Extended-release Capsules, and powder. To a portion of the powder, equivalent to 0.1 g of Diltiazem Hydrochloride, add 100 mL of 0.01 mol/L hydrochloric acid TS, shake thoroughly, and filter. To 1 mL of the filtrate add 0.01 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 234 nm and 238 nm.

Purity Related substances—Take out the content of Diltiazem Hydrochloride Extended-release Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Diltiazem Hydrochloride, add 30 mL of methanol, shake vigorously for 20 minutes, then add methanol to make 50 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than diltiazem obtained from the sample solution is not larger than the peak area of diltiazem obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of diltiazem, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 30 mL. Confirm that the peak area of diltiazem obtained with 20 μL of this solution is equivalent to 4.7 to 8.6% of that obtained with 20 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diltiazem is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Take out the content of 1 capsule of Diltiazem Hydrochloride Extended-release Capsules, add $V/2$ mL of methanol, then add exactly $V/10$ mL of the internal standard solution, and shake vigorously for 20 minutes. Add methanol to make V mL so that each mL contains about 1 mg of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S.HCl$), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 3 mL of the subsequent filtrate, add methanol to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S.HCl$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of diltiazem hydrochloride for assay taken

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 400).

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Take out the content of not less than 20 Diltiazem Hydrochloride Extended-release Capsules, weigh the mass of the content accurately, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of diltiazem hydrochloride ($C_{22}H_{26}N_2O_4S.HCl$), add 50 mL of methanol, then add exactly 10 mL of the internal standard solution, shake vigorously for 20 minutes, and add methanol to make 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, to 3 mL of the subsequent filtrate add methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of diltiazem hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in 50 mL of methanol, add exactly 10 mL of the internal standard solution, and add methanol to make 100 mL. To 3 mL of this solution add methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diltiazem to that of the internal

standard.

$$\text{Amount (mg) of diltiazem hydrochloride } (C_{22}H_{26}N_2O_4S.HCl) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of diltiazem hydrochloride for assay taken

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8 g of sodium acetate trihydrate and 1.5 g of *d*-camphorsulfonic acid in 500 mL of water, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To the filtrate add 250 mL of acetonitrile and 250 mL of methanol.

Flow rate: Adjust so that the retention time of diltiazem is about 9 minutes.

System suitability—

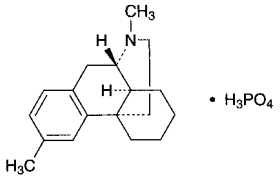
System performance: Dissolve 30 mg of diltiazem hydrochloride, 20 mg of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-on hydrochloride (hereinafter referred to as de-acetyl substance) and 20 mg of phenyl benzoate in methanol to make 200 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, de-acetyl substance, diltiazem and phenyl benzoate are eluted in this order and the resolutions between the peaks of de-acetyl substance and diltiazem and the peaks of diltiazem and phenyl benzoate are not less than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of diltiazem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Dimemorfan Phosphate

ジメモルファンリン酸塩



$C_{18}H_{25}N.H_3PO_4$: 353.39

(9*S*,13*S*,14*S*)-3,17-Dimethylmorphinan monophosphate [*36304-84-4*]

Dimemorfan Phosphate, when dried, contains not less than 98.5% of dimemorfan phosphate ($C_{18}H_{25}N.H_3PO_4$).

Description Dimemorfan Phosphate occurs as white to pale yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 265°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Dimemorfan Phosphate (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelenghs.

(2) Determine the infrared absorption spectrum of Dimemorfan Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibits similar intensities of absorption at the same wave numbers.

(3) To 2 mL of a solution of Dimemorfan Phosphate (1 in 100) add 2 to 3 drops of silver nitrate TS: a yellow precipitate is formed, and it dissolves on the addition of dilute nitric acid.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +25 – +27° (after drying, 1 g, methanol, 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Dimemorfan Phosphate in 100 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Dimemorfan Phosphate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Dimemorfan Phosphate according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Dimemorfan Phosphate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonia solution (28) (150:150:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly the plate with Dragendorff's TS for spraying: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

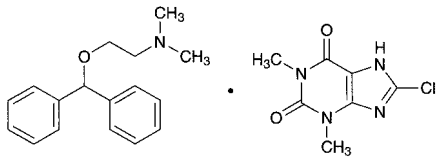
Assay Weigh accurately about 0.6 g of Dimemorfan Phosphate, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 35.34 \text{ mg of } C_{18}H_{25}N.H_3PO_4$$

Containers and storage Containers—Tight containers.

Dimenhydrinate

ジメンヒドリナート



$C_{17}H_{21}NO.C_7H_7ClN_4O_2$: 469.96
2-(Diphenylmethoxy)-*N*,*N*-dimethylethylamine—8-chloro-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (1/1)
[*523-87-5*]

Dimenhydrinate, when dried, contains not less than 53.0% and not more than 55.5% of diphenhydramine ($C_{17}H_{21}NO$: 255.36), and not less than 44.0% and not more than 47.0% of 8-chlorotheophylline ($C_7H_7ClN_4O_2$: 214.61).

Description Dimenhydrinate occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in chloroform, freely soluble in ethanol (95), and slightly soluble in water and in diethyl ether.

Identification **(1)** Dissolve 0.5 g of Dimenhydrinate in 30 mL of dilute ethanol, add 30 mL of water, and use this solution as the sample solution. Transfer 30 mL of the sample solution to a separator, and add 2 mL of ammonia solution (28). Extract with two 10-mL portions of diethyl ether, combine the diethyl ether extracts, wash the combined extracts with 5 mL of water, and then extract the combined extracts with 15 mL of diluted hydrochloric acid (1 in 100). With this acid extract perform the following tests.

(i) To 5 mL of this acid extract add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(ii) To 10 mL of this acid extract add 10 mL of 2,4,6-trinitrophenol TS dropwise, and allow to stand for 30 minutes. Collect the precipitate by filtrating, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt <*2.60*> between 128°C and 133°C.

(2) To 30 mL of the sample solution obtained in (1) add 2 mL of dilute sulfuric acid, and cool for 30 minutes. Scratch the inside wall of the container frequently to facilitate crystallization. Filter, and wash the white crystals with a small amount of ice-cooled water. Dry the crystals for 1 hour at 105°C: the crystals melt <*2.60*> between 300°C and 305°C with decomposition.

(3) To 0.01 g of the crystals obtained in (2) add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. When the dish containing the residue is held over a vessel containing 2 to 3 drops of ammonia TS, the color changes to red-purple, which is discharged on the addition of 2 to 3 drops of sodium hydroxide TS.

(4) Mix well 0.05 g of the crystals obtained in (2) with 0.5 g of sodium peroxide in a nickel crucible, and heat until the mass melts. Cool, dissolve the melted mass in 20 mL of water, and acidify with dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 102 – 107°C

Purity **(1)** Chloride <*1.03*>—Transfer 50 mL of the filtrate obtained in the Assay (2) to a Nessler tube, add 1 mL of nitric acid, and allow to stand for 5 minutes: the turbidity of the solution is not greater than that of the following control solution (not more than 0.044%).

Control solution: Dilute 0.25 mL of 0.01 mol/L hydrochloric acid VS with 6 mL of dilute nitric acid and with water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(2) Bromide and iodide—Place 0.10 g of Dimenhydrinate in a glass-stoppered test tube, and add 0.05 g of sodium nitrite, 10 mL of chloroform and 10 mL of dilute hydrochloric acid. Stopper, shake well, and allow to stand: the chloroform layer remains colorless.

Loss on drying <*2.41*> Not more than 0.5% (3 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <*2.44*> Not more than 0.3% (1 g).

Assay **(1)** Diphenhydramine—Weigh accurately about 0.5 g of Dimenhydrinate, previously dried, transfer to a 250-mL separator, and add 50 mL of water, 3 mL of ammonia TS and 10 g of sodium chloride. Extract with six 15-mL portions of diethyl ether with shaking, combine the diethyl ether extracts, and wash the combined diethyl ether extracts with three 50-mL portions of water. To the diethyl ether extracts add exactly 25 mL of 0.05 mol/L sulfuric acid VS, and add 25 mL of water. Shake thoroughly, and evaporate the diethyl ether gently. Cool, and titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS
= 25.54 mg $C_{17}H_{21}NO$

(2) 8-Chlorotheophylline—Weigh accurately about 0.8 g of Dimenhydrinate, previously dried, transfer to a 200-mL volumetric flask, add 50 mL of water, 3 mL of ammonia TS and 6 mL of a solution of ammonium nitrate (1 in 10), and heat on a water bath for 5 minutes. Add exactly 25 mL of 0.1 mol/L silver nitrate VS, heat on a water bath for 15 minutes with occasional shaking, cool, and add water to make exactly 200 mL. Allow to stand overnight to settle the precipitate, and filter through a dry filter paper, discarding the first 20 mL of the filtrate. Measure exactly 100 mL of the subsequent filtrate, acidify with nitric acid, add 3 mL of nitric acid, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 21.46 mg of $C_7H_7ClN_4O_2$

Containers and storage Containers—Well-closed containers.

Dimenhydrinate Tablets

ジメンヒドリナート錠

Dimenhydrinate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of dimenhydrinate ($C_{17}H_{21}NO.C_7H_7ClN_4O_2$: 469.96).

Method of preparation Prepare as directed under Tablets, with Dimenhydrinate.

Identification **(1)** Triturate a quanity of powdered Dimenhydrinate Tablets, equivalent to 0.5 g of Dimenhydrinate, with 25 mL of warm ethanol (95), and filter.

Dilute the filtrate with 40 mL of water, and filter again. Use the filtrate as the sample solution. Transfer 30 mL of the sample solution to a separator, and proceed as directed in the Identification (1) under Dimenhydrinate.

(2) With 30 mL of the sample solution obtained in (1), proceed as directed in the Identification (2), (3) and (4) under Dimenhydrinate.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Dimenhydrinate Tablets is not less than 85%.

Start the test with 1 tablet of Dimenhydrinate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 28 μg of dimenhydrinate ($C_{17}H_{21}NO.C_7H_7ClN_4O_2$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of dimenhydrinate for assay, previously dried in vacuum using phosphorous (V) oxide as the desiccant for 24 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of dimenhydrinate ($C_{17}H_{21}NO.C_7H_7ClN_4O_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of dimenhydrinate for assay taken
C: Labeled amount (mg) of dimenhydrinate ($C_{17}H_{21}NO.C_7H_7ClN_4O_2$) in 1 tablet

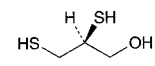
Assay Weigh accurately, and powder not less than 20 Dimenhydrinate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of dimenhydrinate ($C_{17}H_{21}NO.C_7H_7ClN_4O_2$), transfer to a flask, add 40 mL of ethanol (95), and heat with swirling on a water bath until the solution just boils. Continue to heat for 30 seconds, and filter through a glass filter (G4). Wash the filter with warm ethanol (95), transfer the filtrate and washings to a flask, and evaporate the ethanol on a water bath to make 5 mL. Add 50 mL of water, 3 mL of ammonia TS and 6 mL of a solution of ammonium nitrate (1 in 10), heat the mixture on a water bath for 5 minutes, add exactly 25 mL of 0.1 mol/L silver nitrate VS, and heat on a water bath for 15 minutes with occasional shaking. Transfer the mixture to a 200-mL volumetric flask, using water to rinse the flask, cool, add water to make exactly 200 mL, and proceed as directed in the Assay (2) under Dimenhydrinate.

Each mL of 0.1 mol/L silver nitrate VS
$= 47.00$ mg of $C_{17}H_{21}NO.C_7H_7ClN_4O_2$

Containers and storage Containers—Well-closed containers.

Dimercaprol

ジメルカプロール



and enantiomer

$C_3H_8OS_2$: 124.23
(2*RS*)-2,3-Disulfanylpropan-1-ol
[*59-52-9*]

Dimercaprol contains not less than 98.5% and not more than 101.5% of dimercaprol ($C_3H_8OS_2$).

Description Dimercaprol is a colorless or pale yellow liquid. It has a mercaptan-like, disagreeable odor.

It is miscible with methanol and with ethanol (99.5)

It is soluble in peanut oil, and sparingly soluble in water.

It shows no optical rotation.

Identification **(1)** Add 1 drop of Dimercaprol to a mixture of 1 drop of a solution of cobalt (II) chloride hexahydrate (1 in 200) and 5 mL of water: a yellow-brown color develops.

(2) Determine the infrared absorption spectrum of Dimercaprol as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.570 – 1.575

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.238 – 1.248

Purity **(1)** Clarity and color of solution—Dissolve 1.0 mL of Dimercaprol in 20 mL of peanut oil: the solution is clear and colorless to pale yellow.

(2) Bromide—To 2.0 g of Dimercaprol add 25 mL of dilute potassium hydroxide-ethanol TS, and heat in a water bath under a reflux condenser for 2 hours. Evaporate the ethanol in a current of warm air, add 20 mL of water, and cool. Add a mixture of 10 mL of hydrogen peroxide (30) and 40 mL of water, boil gently under a reflux condenser for 10 minutes, and filter rapidly after cooling. Wash the residue with two 10-mL portions of water, combine the washings with the filtrate, add 10 mL of dilute nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS, and titrate ⟨*2.50*⟩ the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination: not more than 1.0 mL of 0.1 mol/L silver nitrate VS is consumed.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Dimercaprol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Assay Weigh accurately about 0.15 g of Dimercaprol into a glass-stoppered flask, dissolve in 10 mL of methanol, and titrate ⟨*2.50*⟩ immediately with 0.05 mol/L iodine VS until a pale yellow color is produced. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 6.212 mg of $C_3H_8OS_2$

Containers and storage Containers—Tight containers.
Storage—Not exceeding 5°C.

Dimercaprol Injection

ジメルカプロール注射液

Dimercaprol Injection is an oily solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of dimercaprol ($C_3H_8OS_2$: 124.23).

Method of preparation Prepare as directed under Injections, with Dimercaprol. Benzyl Benzoate or Benzyl Alcohol may be added to increase the solubility.

Description Dimercaprol Injection is a clear, colorless or light yellow liquid. It has an unpleasant odor.

Identification Measure a volume of Dimercaprol Injection, equivalent to 30 mg of Dimercaprol, and proceed as directed in the Identification (1) under Dimercaprol.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ Perform the test according to Method 2: it meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

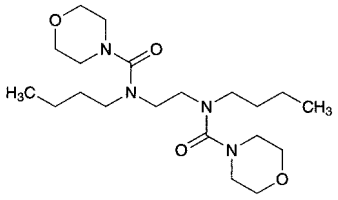
Assay Pipet a volume of Dimercaprol Injection, equivalent to about 0.1 g of dimercaprol ($C_3H_8OS_2$), into a flask, and rinse the pipet several times with a mixture of methanol and diethyl ether (3:1), adding the rinsings to the flask. Add the mixture of methanol and diethyl ether (3:1) to make 50 mL, and titrate ⟨*2.50*⟩ with 0.05 mol/L iodine VS until a yellow color persists. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 6.212 mg of $C_3H_8OS_2$

Containers and storage Containers—Hermetic containers.
Storage—In a cold place.

Dimorpholamine

ジモルホラミン



$C_{20}H_{38}N_4O_4$: 398.54
N,*N*′-Ethylenebis(*N*-butylmorpholine-4-carboxamide)
[*119-48-2*]

Dimorpholamine, when dried, contains not less than 98.0% and not more than 101.0% of dimorpholamine ($C_{20}H_{38}N_4O_4$).

Description Dimorpholamine is a white to light yellow, crystalline powder, masses or syrupy liquid.

It is very soluble in ethanol (99.5) and in acetic anhydride, and soluble in water.

The pH of a solution prepared by dissolving 1.0 g of Dimorpholamine in 10 mL of water is between 6.0 and 7.0.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Dimorpholamine (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dimorpholamine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Dimorpholamine in 50 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride ⟨*1.03*⟩—To 20 mL of the solution obtained in (1) add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Sulfate ⟨*1.14*⟩—To 10 mL of the solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.096%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Dimorpholamine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.20 g of Dimorpholamine in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5) and water (4:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 10 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 8 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Dimorpholamine, previously dried, dissolve in 50 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.85 mg of $C_{20}H_{38}N_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dimorpholamine Injection

ジモルホラミン注射液

Dimorpholamine Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of dimorpholamine ($C_{20}H_{38}N_4O_4$: 398.54).

Method of preparation Prepare as directed under Injections, with Dimorpholamine.

Description Dimorpholamine Injection is a clear, colorless liquid.

pH: 3.0 – 5.5

Identification **(1)** To a volume of Dimorpholamine Injection, equivalent to 0.1 g of Dimorpholamine, add 3 drops of Dragendorff's TS: an orange color develops.

(2) To a volume of Dimorpholamine Injection, equivalent to 50 mg of Dimorpholamine, add 1 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 2 mL of hydrochloric acid, boil for 10 minutes under a reflux condenser, and evaporate to dryness on a water bath. Dissolve the residue with 1 mL of water, neurtralize with sodium hydroxide TS, and add 0.2 mL of a solution of acetaldehyde (1 in 20), 0.1 mL of sodium pentacyanonitrosyl ferrate (III) TS and 0.5 mL of sodium carbonate TS: a blue color develops.

Bacterial endotoxins ⟨*4.01*⟩ Less than 5.0 EU/mg. Perform the test with the sample diluted to 0.15 w/v% with water for bacterial endotoxins test.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Dimorpholamine Injection, equivalent to about 30 mg of dimorpholamine ($C_{20}H_{38}N_4O_4$), and add water to make exactly 200 mL. Pipet 1 mL of this solution, shake with exactly 4 mL of the internal standard solution for 5 minutes, and use this solution as the sample solution. Separately, weigh accurately about 0.15 g of dimorpholamine for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 8 hours, and dissolve in water to make exactly 1000 mL. Pipet 1 mL of this solutionn, shake with exactly 4 mL of the internal standard solution for 5 minutes, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dimorpholamine to that of the inernal standard.

$$\text{Amount (mg) of dimorpholamine } (C_{20}H_{38}N_4O_4) = M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of dimorpholamine for assay taken

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 25,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 216 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of dimorpholamine is about 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, dimorpholamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

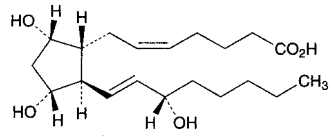
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dimorpholamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Dinoprost

Prostaglandin F_{2a}

ジノプロスト



$C_{20}H_{34}O_5$: 354.48

(5*Z*)-7-{(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-en-1-yl]cyclopentyl}hept-5-enoic acid

[*551-11-1*]

Dinoprost contains not less than 98.5% of dinoprost ($C_{20}H_{34}O_5$), calculated on the anhydrous basis.

Description Dinoprost occurs as white, waxy masses or powder, or a clear, colorless to light yellow and viscous liquid. It is odorless.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and very slightly soluble in water.

Identification **(1)** To 5 mg of Dinoprost add 2 mL of sulfuric acid, and dissolve by shaking for 5 minutes: a dark red color develops. To this solution add 30 mL of sulfuric acid: an orange color develops with a green fluorescence.

(2) Dissolve 1 mg of Dinoprost in 50 mL of diluted sulfuric acid (7 in 10), and warm in a water bath warmed at 50°C for 40 minutes. After cooling, determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Warm Dinoprost at 40°C to effect a liquid, and determine the infrared absorption spectrum of the liquid as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibits similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +24 – +31° (0.2 g, ethanol (99.5), 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Dinoprost in 5 mL of ethanol (99.5): the solution is clear and colorless to pale yellow.

(2) Related substances—Dissolve 10 mg of Dinoprost in 2 mL of methanol, add water to make 10 mL, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than dinoprost from the sample solution is not larger than the peak area of dinoprost from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogenphosphate TS and acetonitrile (5:2).

Flow rate: Adjust so that the retention time of dinoprost is about 20 minutes.

Selection of column: Dissolve 0.01 g each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 2 mL of methanol, and add water to make 10 mL. To 1 mL of this solution add diluted methanol (1 in 5) to make 30 mL, proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dinoprost from the standard solution composes 5% to 15% of the full scale.

Time span of measurement: About 1.5 times as long as the retention time of dinoprost, beginning after the solvent peak.

Water ⟨*2.48*⟩ Not more than 0.5% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately about 50 mg of Dinoprost, dissolve in 30 mL of *N*,*N*-dimethylformamide, and titrate ⟨*2.50*⟩ with 0.02 mol/L tetramethylammonium hydroxide VS under a stream of nitrogen (potentiometric titration). Perform a blank determination, and make any necessary correction.

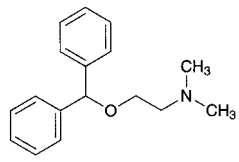
Each mL of 0.02 mol/L tetramethylammonium hydroxide VS
= 7.090 mg of $C_{20}H_{34}O_5$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a place not exceeding 5°C.

Diphenhydramine

ジフェンヒドラミン



$C_{17}H_{21}NO$: 255.35
2-(Diphenylmethoxy)-*N*,*N*-dimethylethylamine
[*58-73-1*]

Diphenhydramine contains not less than 96.0% of diphenhydramine ($C_{17}H_{21}NO$).

Description Diphenhydramine is a clear, light yellow to yellow liquid. It has a characteristic odor, and has a burning taste at first, followed by a slight sensation of numbness on the tongue.

It is miscible with acetic anhydride, with acetic acid (100), with ethanol (95) and with diethyl ether.

It is very slightly soluble in water.

Boiling point: about 162°C (in vacuum, 0.67 kPa).

Refractive index n_D^{20}: about 1.55

It is gradually affected by light.

Identification **(1)** To 0.05 g of Diphenhydramine add 2 mL of sulfuric acid: an orange-red precipitate is produced immediately, and its color changes to red-brown on standing. Add carefully 2 mL of water to this solution: the intensity of the color changes, but the color tone does not change.

(2) Dissolve 0.1 g of Diphenhydramine in 10 mL of dilute ethanol, add an excess of a saturated solution of 2,4,6-trinitrophenol in dilute ethanol with stirring, and cool in ice. Collect the produced crystals, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt ⟨*2.60*⟩ between 128°C and 133°C.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.013 – 1.020

Purity **(1)** β-Dimethylaminoethanol—Dissolve 1.0 g of Diphenhydramine in 20 mL of diethyl ether, and extract with two 10-mL portions of water with thorough shaking. Combine the water extracts, and add 2 drops of phenolphthalein TS and 1.0 mL of 0.05 mol/L sulfuric acid VS: no red color develops.

(2) Benzohydrol—Transfer 1.0 g of Diphenhydramine to a separator, dissolve in 20 mL of diethyl ether, and extract with two 25-mL portions of diluted hydrochloric acid (1 in 15) with thorough shaking. Separate the diethyl ether layer, evaporate slowly on a water bath, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the mass of the residue is not more than 20 mg.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Diphenhydramine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

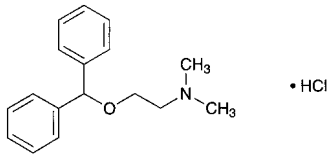
Assay Weigh accurately about 0.5 g of Diphenhydramine, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.54 mg of $C_{17}H_{21}NO$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and almost well-filled.

Diphenhydramine Hydrochloride

ジフェンヒドラミン塩酸塩



$C_{17}H_{21}NO.HCl$: 291.82
2-(Diphenylmethoxy)-*N*,*N*-dimethylethylamine monohydrochloride
[*147-24-0*]

Diphenhydramine Hydrochloride, when dried, contains not less than 98.0% of diphenhydramine hydrochloride ($C_{17}H_{21}NO.HCl$).

Description Diphenhydramine Hydrochloride occurs as white, crystals or crystalline powder. It is odorless, and has a bitter taste, followed by a sensation of numbness on the tongue.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually affected by light.

Identification **(1)** Determine the absorption spectrum of a solution of Diphenhydramine Hydrochloride in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dipenhydramine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Diphenhydramine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> Dissolve 1.0 g of Diphenhydramine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

Melting point <*2.60*> 166 – 170°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Diphenhydramine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Diphenhydramine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Diphenhydramine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate, methanol and ammonia solution (28) (10:4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iodine TS on the plate: the spots other than the principal spot and the spot on the original point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (2 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Diphenhydramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.18 mg of $C_{17}H_{21}NO.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Diphenhydramine and Bromovalerylurea Powder

ジフェンヒドラミン·バレリル尿素散

Method of preparation

Diphenhydramine Tannate	90 g
Bromovalerylurea	500 g
Starch, Lactose Hydrate, or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Diphenhydramine and Bromovalerylurea Powder occurs as a slightly grayish white powder.

Identification **(1)** To 0.1 g of Diphenhydramine and Bromovalerylurea Powder add 5 mL of dilute hydrochloric acid, 1 mL of ethanol (95) and 10 mL of water, shake, and filter. To the filtrate add 10 mL of sodium hydroxide TS, and extract with 10 mL of chloroform. Separate the chloroform layer, add 1 mL of bromophenol blue TS, and shake: a yellow color develops in the chloroform layer (diphenhydramine tannate).

(2) Shake 0.02 g of Diphenhydramine and Bromovalerylurea Powder with 10 mL of diethyl ether, filter, and evaporate the filtrate on a water bath. Dissolve the residue in 2 mL of sodium hydroxide TS, and add 5 mL of dimethylglyoxime-thiosemicarbazide TS, and heat on a water bath for 30 minutes: a red color develops (bromovalerylurea).

(3) Shake 0.3 g of Diphenhydramine and Bromovalerylurea Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Dissolve 0.15 of bromovalerylurea and 0.03 g of diphenhydramine tannate in 5 mL each of methanol, and use the solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluores-

cent indicator for thin-layer chromatography. Develop the plate in a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm. Air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): 3 spots from the sample solution and the corresponding spot from standard solutions (1) and (2) show the same *R*f value. Spray Dragendorff's TS for spraying evenly on the plate: the spot from the standard solution (2) and the corresponding spot from the sample solution reveal an orange color.

Containers and storage Containers—Well-closed containers.

Diphenhydramine, Phenol and Zinc Oxide Liniment

ジフェンヒドラミン·フェノール·亜鉛華リニメント

Method of preparation

Diphenhydramine	20 g
Phenol and Zinc Oxide Liniment	980 g
To make	1000 g

Dissolve and mix the above ingredients.

Description Diphenhydramine, Phenol and Zinc Oxide Liniment is a white to whitish, pasty mass. It has a slight odor of phenol.

Identification (1) To 3 g of Diphenhydramine, Phenol and Zinc Oxide Liniment add 20 mL of hexane, shake well, and separate the hexane layer. Shake thoroughly the hexane solution with 10 mL of 0.2 mol/L hydrochloric acid. Separate the aqueous layer, and adjust with sodium hydroxide TS to a pH of 4.6. Add 1 mL of bromophenol blue-potassium biphthalate TS and 10 mL of chloroform, and shake: a yellow color develops in the chloroform layer (diphenhydramine).

(2) Place 1 g of Diphenhydramine, Phenol and Zinc Oxide Liniment in a porcelain crucible, gradually raise the temperature by heating until the mass is charred, and ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. Add 2 to 3 drops of potassium hexacyanoferrate (II) TS to the filtrate: a white precipitate is produced (zinc oxide).

(3) Shake 0.5 g of Diphenhydramine, Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, filter, and use the filtrate as the sample solution. Dissolve 0.01 g each of diphenhydramine and phenol in 5 mL each of chloroform, and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: two spots from the sample solution and each spot from the standard solution (1) and (2) show the same *R*f value. Sublime iodine, and spray Dragendorff's TS evenly upon the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal an orange color.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Diphenhydramine Tannate

タンニン酸ジフェンヒドラミン

Diphenhydramine Tannate is a compound of diphenhydramine and tannic acid.

It contains not less than 25.0% and not more than 35.0% of diphenhydramine ($C_{17}H_{21}NO$: 255.35).

Description Diphenhydramine Tannate occurs as a grayish white to light brown powder. It is odorless or has a slight, characteristic odor. It is tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) To 1 g of Diphenhydramine Tannate add 15 mL of water and 0.3 mL of dilute hydrochloric acid, shake thoroughly for 1 minute, filter, and use this filtrate as the sample solution. Transfer 10 mL of the sample solution to a separator, extract with two 20-mL portions of chloroform, combine the chloroform extracts, and evaporate on a water bath to dryness. To 5 mL of a solution of the residue (1 in 100) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(2) To 10 mL of a solution of the residue obtained in (1) (1 in 100) add 10 mL of 2,4,6-trinitrophenol TS dropwise, and allow to stand for 30 minutes. Collect the precipitate by filtration, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt <*2.60*> between 128°C and 133°C.

(3) To 1 mL of the sample solution obtained in (1) add 1 drop of iron (III) chloride TS: a dark blue-purple color develops.

Purity Heavy metals <*1.07*>—Proceed with 1.0 g of Diphenhydramine Tannate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <*2.41*> Not more than 7.0% (1 g, 105°C, 5 hours).

Residue on ignition <*2.44*> Not more than 1.0% (1 g).

Assay Transfer about 1.7 g of Diphenhydramine Tannate, accurately weighed, to a separator, dissolve in 20 mL of water and 3.0 mL of dilute hydrochloric acid with thorough shaking, add 20 mL of a solution of sodium hydroxide (1 in 10) and exactly 25 mL of isooctane, shake vigorously for 5 minutes, dissolve 2 g of sodium chloride with shaking, and allow to stand. To 20 mL of the isooctane layer add exactly 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.54 mg of $C_{17}H_{21}NO$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Freeze-dried Diphtheria Antitoxin, Equine

乾燥ジフテリアウマ抗毒素

Freeze-dried Diphtheria Antitoxin, Equine, is a preparation for injection which is dissolved before use.

It contains diphtheria antitoxin in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Diphtheria Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Diphtheria Antitoxin, Equine, becomes a colorless or light yellow-brown, clear liquid or a slightly whitish turbid liquid on addition of solvent.

Diphtheria Toxoid

ジフテリアトキソイド

Diphtheria Toxoid is a liquid for injection containing diphtheria toxoid prepared by treating diphtheria toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity.

It conforms to the requirements of Diphtheria Toxoid in the Minimum Requirements for Biological Products.

Description Diphtheria Toxoid is a clear, colorless to light yellow-brown liquid.

Adsorbed Diphtheria Toxoid for Adult Use

成人用沈降ジフテリアトキソイド

Adsorbed Diphtheria Toxoid for Adult Use is a liquid for injection containing diphtheria toxoid prepared by treating diphtheria toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity and very few antigenic substances other than toxoid, and rendered insoluble with aluminum salt.

It conforms to the requirements of Adsorbed Diphtheria Toxoid for Adult Use in the Minimum Requirements of Biological Products.

Description Adsorbed Diphtheria Toxoid for Adult Use becomes a homogeneous, whitish turbid liquid on shaking.

Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine

沈降精製百日せきジフテリア破傷風混合ワクチン

Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine is a liquid for injection consisting of a liquid containing the protective antigen of *Bordetella pertussis*, Diphtheria Toxoid and a liquid containing tetanus toxoid obtained by detoxifying the tetanus toxin with formaldehyde solution without impairing its immunogenicity, to which aluminum is added to make the antigen and the toxoids insoluble.

It conforms to the requirements of Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine becomes a homogeneous, white turbid liquid on shaking.

Diphtheria-Tetanus Combined Toxoid

ジフテリア破傷風混合トキソイド

Diphtheria-Tetanus Combined Toxoid is a liquid for injection containing diphtheria toxoid and tetanus toxoid which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method involving no appreciable loss of the immunogenicity.

It conforms to the requirements of Diphtheria-Tetanus Combined Toxoid in the Minimum Requirements of Biological Products.

Description Diphtheria-Tetanus Combined Toxoid is a colorless or light yellow-brown, clear liquid.

Adsorbed Diphtheria-Tetanus Combined Toxoid

沈降ジフテリア破傷風混合トキソイド

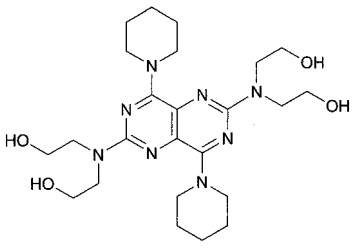
Adsorbed Diphtheria-Tetanus Combined Toxoid is a liquid for injection containing diphtheria toxoid and tetanus toxoid which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by adding aluminum salt.

It conforms to the requirements of Adsorbed Diphtheria-Tetanus Combined Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Diphtheria-Tetanus Combined Toxoid becomes a homogeneous, whitish turbid liquid on shaking.

Dipyridamole

ジピリダモール



$C_{24}H_{40}N_8O_4$: 504.63
2,2′,2″,2‴-{[4,8-Di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl]dinitrilo}tetraethanol
[*58-32-2*]

Dipyridamole, when dried, contains not less than 98.5% of dipyridamole ($C_{24}H_{40}N_8O_4$).

Description Dipyridamole occurs as yellow, crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water and in diethyl ether.

Identification **(1)** Dissolve 5 mg of Dipyridamole in 2 mL of sulfuric acid, add 2 drops of nitric acid, and shake: a deep purple color develops.

(2) Determine the absorption spectrum of a solution of Dipyridamole in a mixture of methanol and hydrochloric acid (99:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Dipyridamole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 165 – 169°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Dipyridamole in 10 mL of chloroform: the solution is clear, and shows a yellow color.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Dipyridamole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Dipyridamole according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Dipyridamole in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than dipyridamole from the sample solution is not larger than the peak area of dipyridamole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.2 g of potassium dihydrogen phosphate in 200 mL of water, and add 800 mL of methanol.

Flow rate: Adjust so that the retention time of dipyridamole is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of dipyridamole.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of dipyridamole obtained from 20 μL of this solution is equivalent to 15 to 25% of that of dipyridamole obtained from 20 μL of the standard solution.

System performance: Dissolve 7 mg of Dipyridamole and 3 mg of terphenyl in 50 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, dipyridamole and terphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dipyridamole is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.2% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Dipyridamole, previously dried, dissolve in 70 mL of methanol, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

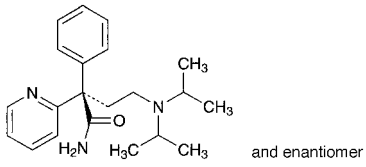
Each mL of 0.1 mol/L perchloric acid VS
= 50.46 mg of $C_{24}H_{40}N_8O_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Disopyramide

ジソピラミド



and enantiomer

$C_{21}H_{29}N_3O$: 339.47
(2*RS*)-4-Bis(1-methylethyl)amino-2-phenyl-2-(pyridin-2-yl)butanamide
[*3737-09-5*]

Disopyramide contains not less than 98.5% of disopyramide ($C_{21}H_{29}N_3O$), calculated on the dried basis.

Description Disopyramide occurs as white, crystals or crystalline powder.

It is very soluble in methanol and in ethanol (95), freely soluble in acetic anhydride, in acetic acid (100) and in diethyl ether, and slightly soluble in water.

Identification **(1)** To 1 mL of a solution of Disopyramide in ethanol (95) (1 in 20) add 10 mL of 2,4,6-trinitrophenol TS, and warm: a yellow precipitate is formed. Filter this precipitate, wash with water, and dry at 105°C for 1 hour: the residue melts ⟨*2.60*⟩ between 172°C and 176°C.

(2) Determine the absorption spectrum of a solution of Disopyramide in 0.05 mol/L sulfuric acid-methanol TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Disopyramide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (269 nm): 194 – 205 (10 mg, 0.05 mol/L sulfuric acid-methanol TS, 500 mL).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Disopyramide in 10 mL of ethanol (95), and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 10 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Disopyramide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.40 g of Disopyramide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 400 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and ammonia solution (28) (45:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, 80°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

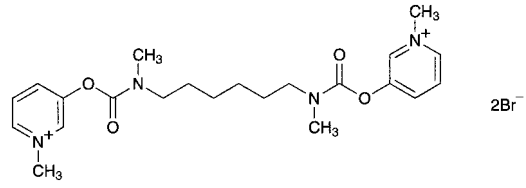
Assay Weigh accurately about 0.25 g of Disopyramide, dissolve in 30 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.97 mg of $C_{21}H_{29}N_3O$

Containers and storage Containers—Tight containers.

Distigmine Bromide

ジスチグミン臭化物



$C_{22}H_{32}Br_2N_4O_4$: 576.32
3,3′-[Hexamethylenebis(methyliminocarbonyloxy)]bis(1-methylpyridinium) dibromide
[*15876-67-2*]

Distigmine Bromide contains not less than 98.5% of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$), calculated on the anhydrous basis.

Description Distigmine Bromide occurs as a white crystalline powder.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), and slightly soluble in acetic anhydride.

The pH of a solution of Distigmine Bromide (1 in 100) is between 5.0 and 5.5.

It is slightly hygroscopic.

It is gradually colored by light.

Melting point: about 150°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Distigmine Bromide (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Distigmine Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Distigmine Bromide (1 in 10) add 2 mL of dilute nitric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (1) for bromide.

Purity **(1)** Clarity and color of solution—Dissolve 0.25 g of Distigmine Bromide in 5 mL of water: the solution is clear and colorless.

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.40 g of Dis-

tigmine Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Distigmine Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 40 mg of Distigmine Bromide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, ethanol (99.5) and acetic acid (100) (8:3:2:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 1.0% (1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Distigmine Bromide, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (8:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration with platinum electrode). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.82 mg of $C_{22}H_{32}Br_2N_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Distigmine Bromide Tablets

ジスチグミン臭化物錠

Distigmine Bromide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$: 576.32).

Method of preparation Prepare as directed under Tablets, with Distigmine Bromide.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 268 nm and 272 nm, and a minimum between 239 nm and 243 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Distigmine Bromide Tablets add 30 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, and add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 30 μg of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$)
$= M_S \times (A_{T2} - A_{T1})/(A_{S2} - A_{S1}) \times V'/V \times 1/20$

M_S: Amount (mg) of distigmine bromide for assay taken, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method, using 500 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Distigmine Bromide Tablets is not less than 80%.

Start the test with 1 tablet of Distigmine Bromide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 10 μg of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of distigmine bromide for assay (separately determine the water <*2.48*> in the same manner as Distigmine Bromide), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 500 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_{T1} and A_{S1}, at 270 nm, and A_{T2} and A_{S2}, at 350 nm.

Dissolution rate (%) with respect to the labeled amount of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$)
$= M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times V'/V \times 1/C \times 10$

M_S: Amount (mg) of distigmine bromide for assay taken, calculated on the anhydrous basis
C: Labeled amount (mg) of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 tablets of Distigmine Bromide Tablets. Weigh accurately a portion of the powder, equivalent to about 15 mg of Distigmine Bromide ($C_{22}H_{32}Br_2N_4O_4$), add 30 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and filter. Discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of distigmine bromide for assay (previously determine the water <*2.48*> in the same manner as Distigmine Bromide), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution, A_{T2} and A_{S2}, at 270 nm and, A_{T1} and A_{S1}, at 241 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, respectively.

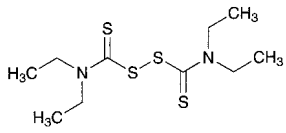
Amount (mg) of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$)
$= M_S \times (A_{T2} - A_{T1})/(A_{S2} - A_{S1}) \times 1/2$

M_S: Amount (mg) of distigmine bromide for assay taken, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Disulfiram

ジスルフィラム



$C_{10}H_{20}N_2S_4$: 296.54
Tetraethylthiuram disulfide
[*97-77-8*]

Disulfiram, when dried, contains not less than 99.0% of disulfiram ($C_{10}H_{20}N_2S_4$).

Description Disulfiram occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in acetone and in toluene, sparingly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Disulfiram in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Disulfiram, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 70 – 73°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Disulfiram according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Disulfiram according to Method 4, and perform the test (not more than 2 ppm).

(3) Diethyldithiocarbamic acid—Dissolve 0.10 g of Disulfiram in 10 mL of toluene, and shake with 10 mL of diluted sodium carbonate TS (1 in 20). Discard the toluene layer, wash the water layer with 10 mL of toluene, shake with 5 drops of a solution of cupric sulfate (1 in 250) and 2 mL of toluene, and allow to stand: no light yellow color develops in the toluene layer.

(4) Related substances—Dissolve 50 mg of Disulfiram in 40 mL of methanol, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than disulfiram from the sample solution is not larger than the peak area of disulfiram from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust so that the retention time of disulfiram is about 8 minutes.

Selection of column: Dissolve 50 mg of Disulfiram and 50 mg of benzophenone in 40 mL of methanol, and add water to make 50 mL. To 1 mL of this solution add the mobile phase to make 200 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of benzophenone and disulfiram in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust so that the peak height of disulfiram obtained from 10 μL of the standard solution is 15 – 30 mm.

Time span of measurement: About 3.5 times of the retention time of disulfiram.

Loss on drying <*2.41*> Not more than 0.20% (2 g, silica gel, 24 hours).

Residue on ignition <*2.44*> Not more than 0.1% (2 g).

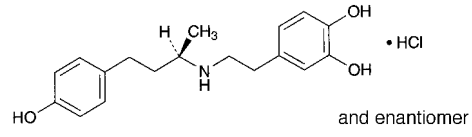
Assay Weigh accurately about 0.2 g of Disulfiram, previously dried, in an iodine bottle, dissolve in 20 mL of acetone, add 1.5 mL of water and 1.0 g of potassium iodide, and dissolve by shaking thoroughly. To this solution add 3.0 mL of hydrochloric acid, stopper the bottle tightly, shake, and allow to stand in a dark place for 3 minutes. Add 70 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 14.83 mg of $C_{10}H_{20}N_2S_4$

Containers and storage Containers—Tight containers.

Dobutamine Hydrochloride

ドブタミン塩酸塩



$C_{18}H_{23}NO_3$.HCl: 337.84
4-{2-[(1*RS*)-3-(4-Hydroxyphenyl)-1-methylpropylamino]ethyl}benzene-1,2-diol monohydrochloride
[*49745-95-1*]

Dobutamine Hydrochloride, when dried, contains not less than 98.0% of dobutamine hydrochloride ($C_{18}H_{23}NO_3$.HCl).

Description Dobutamine Hydrochloride occurs as white to very pale orange, crystalline powder or grains.

It is freely soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Dobutamine Hydrochloride (1 in 100) shows no optical rotation.

Identification **(1)** Determine the infrared absorption spectra of Dobutamine Hydrochloride, previously dried, as di-

rected in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Dobutamine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Dobutamine Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨1.09⟩ (2) for chloride.

pH ⟨2.54⟩ Dissolve 1.0 g of Dobutamine Hydrochloride in 100 mL of water: the pH of this solution is between 4.0 and 5.5.

Melting point ⟨2.60⟩ 188 – 192°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Dobutamine Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨1.07⟩—Dissolve 1.0 g of Dobutamine Hydrochloride in 40 mL of water by warming, cool, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Dobutamine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and formic acid (78:22:5) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨2.41⟩ Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g each of Dobutamine Hydrochloride and Dobutamine Hydrochloride RS, each previously dried, dissolve separately in exactly 10 mL of the internal standard solution, add diluted methanol (1 in 2) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dobutamine to that of the internal standard, respectively.

$$\text{Amount (mg) of dobutamine hydrochloride } (C_{18}H_{23}NO_3.HCl) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Dobutamine Hydrochloride RS taken

Internal standard solution—A solution of salicylamide in diluted methanol (1 in 2) (1 in 125).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of tartrate buffer solution (pH 3.0) and methanol (7:3).

Flow rate: Adjust so that the retention time of dobutamine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, dobutamine and internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dobutamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Docetaxel Hydrate

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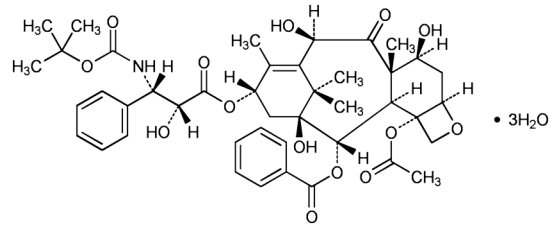

$C_{43}H_{53}NO_{14}.3H_2O$: 861.93

(1*S*,2*S*,3*R*,4*S*,5*R*,7*S*,8*S*,10*R*,13*S*)-4-Acetoxy-2-benzoyloxy-5,20-epoxy-1,7,10-trihydroxy-9-oxotax-11-en-13-yl (2*R*,3*S*)-3-(1,1-dimethylethyl)oxycarbonylamino-2-hydroxy-3-phenylpropanoate trihydrate

[*148408-66-6*]

Docetaxel Hydrate contains not less than 97.5% and not more than 102.0% of docetaxel ($C_{43}H_{53}NO_{14}$: 807.88), calculated on the anhydrous and residual solvent-free basis.

Description Docetaxel Hydrate occurs as a white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide and in ethanol (99.5), soluble in methanol and in dichloromethane, and practically insoluble in water.

It decomposes on exposure to light.

Identification **(1)** Determine the absorption spectrum of a solution of Docetaxel Hydrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Docetaxel Hydrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 60 mg of Docetaxel Hydrate in 1 mL of dichloromethane. Perform the test with this solution as directed in the solution method under Infrared Spectrophotometry ⟨2.25⟩ using a fixed cell composed of potassium bromide optical plates with the cell length of 0.1 mm, and compare the spectrum with the Reference Spectrum or the spectrum of Docetaxel Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨2.49⟩ $[\alpha]_D^{20}$: −39 – −41° (0.2 g calculated

on the anhydrous and residual solvent-free basis, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Docetaxel Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay, as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak, having the relative retention time of about 0.97, about 1.08, and about 1.13 to docetaxel, is not more than 0.50%, not more than 0.30%, and not more than 0.30%, respectively, the amount of each peak other than docetaxel and the peaks mentioned above is not more than 0.10%, and the total amount of the peaks other than docetaxel is not more than 1.0%. For the area of the peak, having the relative retention time of about 0.97 to docetaxel, multiply the relative response factor 1.6.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: For 39 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 100 mL. To 1 mL of this solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 10 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 10 mL. Confirm that the peak area of docetaxel obtained with 10 μL of this solution is equivalent to 35 to 65% of that obtained with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 2.0%.

Water <*2.48*> 5.0 – 7.0% (50 mg, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Docetaxel Hydrate and Docetaxel RS (separately determine the water <*2.48*> and the residual solvent in the same manner as Docetaxel Hydrate), dissolve them separately in 2.5 mL of ethanol (99.5), add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of docetaxel in each solution.

Amount (mg) of docetaxel ($C_{43}H_{53}NO_{14}$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Docetaxel RS taken, calculated on the anhydrous and residual solvent-free basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase A: Water.

Mobile phase B: Acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 9	72	28
9 – 39	72 → 28	28 → 72

Flow rate: 1.2 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Docetaxel Injection

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Docetaxel Injection is a hydrophilic injection.

It contains not more than 93.0% and not less than 105.0% of the labeled amount of docetaxel ($C_{43}H_{53}NO_{14}$: 807.88).

Method of preparation Prepare as directed under Injections, with Docetaxel Hydrate.

Description Docetaxel Injection occurs as a clear and pale yellow to yellowish orange, liquid.

Identification To a volume of Docetaxel Injection, equivalent to 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 50 mL of methanol, and use this solution as the sample solution. Separately, dissolve 4 mg of docetaxel hydrate in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Then develop the plate with a mixture of ethyl acetate, heptane and ethanol (99.5) (12:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the spot from the sample solution and the standard solution is the same.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay, as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak, having the relative retention time of about 0.27, about 1.05, about 1.08, about 1.13, and about 1.18 to docetaxel, is not more than 0.30%, not more than 1.3%, not more than 1.5%, not more than 0.50%, and not more than 0.50%, respectively, the amount of each peak other than docetaxel, the peak having the relative retention time of about 0.97 and the peaks mentioned above is not more than 0.20%, and the total amount of the peaks other than docetaxel and the peak having the relative retention time of about 0.97 is not more than 3.5%. For the area of the peak, having the relative retention time of about 0.27 to docetaxel, multiply the relative response factor 0.67.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

Time span of measurement: For 39 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL. Confirm that the peak area of docetaxel obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 2.0%.

Bacterial endotoxins <4.01> Less than 2.5 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To exactly a volume of Docetaxel Injection, equivalent to about 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 5 mL of ethanol (99.5), further add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Docetaxel RS (separately determine the water <2.48> and the residual solvent in the same manner as Docetaxel Hydrate), dissolve in 20 mL of ethanol (99.5), add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of docetaxel in each solution.

$$\text{Amount (mg) of docetaxel } (C_{43}H_{53}NO_{14}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Docetaxel RS taken, calculated on the anhydrous and residual solvent-free basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Docetaxel for Injection

注射用ドセタキセル

Docetaxel for Injection is a preparation for injection which is dissolved before use.

It contains not more than 93.0% and not less than 105.0% of the labeled amount of docetaxel ($C_{43}H_{53}NO_{14}$: 807.88).

Method of preparation Prepare as directed under Injections, with Docetaxel Hydrate.

Description Docetaxel for Injection occurs as a clear and yellow to orange-yellow, viscous liquid.

Identification To an amount of Docetaxel for Injection, equivalent to 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 50 mL of methanol, and use this solution as the sample solution. Separately, dissolve 4 mg of docetaxel hydrate in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Then develop the plate with a mixture of ethyl acetate, heptane and ethanol (99.5) (12:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the spot obtained from the sample solution and the standard solution is the same.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay, as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic in-

tegration method, and calculate the amount of them by the area percentage method: the amount of each peak, having the relative retention time of about 0.27, about 1.05, about 1.08, about 1.13, and about 1.18 to docetaxel, is not more than 0.30%, not more than 1.3%, not more than 1.5%, not more than 0.50%, and not more than 0.50%, respectively, the amount of each peak other than docetaxel, the peak having the relative retention time of about 0.97 and the peaks mentioned above is not more than 0.20%, and the total amount of the peaks other than docetaxel and the peak having the relative retention time of about 0.97 is not more than 3.5%. For the area of the peak, having the relative retention time of about 0.27 to docetaxel, multiply the relative response factor 0.67.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

Time span of measurement: For 39 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL. Confirm that the peak area of docetaxel obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 2.0%.

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.5 EU/mg.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test. (*T*: 120.0%).

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately an amount of Docetaxel for Injection, equivalent to about 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 5 mL of ethanol (99.5), further add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Docetaxel RS (separately determine the water ⟨*2.48*⟩ and the residual solvent in the same manner as Docetaxel Hydrate), dissolve in 20 mL of ethanol (99.5), add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of docetaxel in each solution.

Amount (mg) of docetaxel ($C_{43}H_{53}NO_{14}$) in 1 mL of Docetaxel for Injection
$= M_S/M_T \times A_T/A_S \times d \times 1/2$

M_S: Amount (mg) of Docetaxel RS taken, calculated on the anhydrous and residual solvent-free basis
M_T: Amount (mg) of Docetaxel for Injection taken
d: Density (g/mL) of Docetaxel for Injection

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Domperidone

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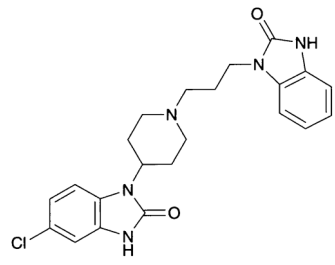

$C_{22}H_{24}ClN_5O_2$: 425.91

5-Chloro-1-{1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]piperidin-4-yl}-1,3-dihydro-2*H*-benzimidazol-2-one

[*57808-66-9*]

Domperidone, when dried, contains not less than 99.0% and not more than 101.0% of domperidone ($C_{22}H_{24}ClN_5O_2$).

Description Domperidone occurs as a white to pale yellow, crystalline powder or powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), very slightly soluble in 2-propanol, and practically insoluble in water.

Melting point: about 243°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Domperidone in a mixture of 2-propanol and 0.1 mol/L hydrochloric acid TS (9:1) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Domperidone as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and com-

pare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Domperidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Domperidone in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than domperidone obtained from the sample solution is not larger than 1/2 times the peak area of domperidone obtained from the standard solution. Furthermore, the total area of the peaks other than domperidone is not larger than the peak area of domperidone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 287 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 of this solution with a solution prepared by dissolving 2.31 g of phosphoric acid in water to make 1000 mL. To 500 mL of this solution add 500 mL of methanol.

Flow rate: Adjust so that the retention time of domperidone is about 9 minutes.

Time span of measurement: About 4 times as long as the retention time of domperidone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 5 mL. Confirm that the peak area of domperidone obtained from 10 μL of this solution is equivalent to 30 to 50% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 10 mg of Domperidone and 20 mg of ethyl parahydroxybenzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, domperidone and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of domperidone is not more than 3.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Domperidone, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.59 mg of $C_{22}H_{24}ClN_5O_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Donepezil Hydrochloride

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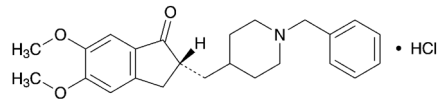


and enantiomer

$C_{24}H_{29}NO_3.HCl$: 415.95

(2*RS*)-2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one monohydrochloride

[*120011-70-3*]

Donepezil Hydrochloride contains not less than 98.0% and not more than 102.0% of donepezil hydrochloride ($C_{24}H_{29}NO_3.HCl$), calculated on the anhydrous basis.

Description Donepezil Hydrochloride occurs as a white crystalline powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

A solution of Donepezil Hydrochloride (1 in 100) shows no optical rotation.

Donepezil Hydrochloride shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Donepezil Hydrochloride (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Donepezil Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Donepezil Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Donepezil Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Donepezil Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Purity **(1)** Heavy metals <*1.07*>—To 1.0 g of Donepezil Hydrochloride in a porcelain or platinum crucible add 5 mL of sulfuric acid, incinerate by heating gradually, then incinerate by ignition between 500 and 600°C. If a carbonized residue still retains, moisten the residue with a little amount of sulfuric acid, and incinerate again by ignition between 500 and 600°C. After cooling, dissolve the residue with 3 mL of hydrochloric acid, then evaporate to dryness on a water bath or hot plate, and dissolve the residue with 10 mL of water by warming. Then, proceed as directed in Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Donepezil Hydrochloride in 25 mL of the mobile phase. To 10 mL of

this solution add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than donepezil obtained from the sample solution is not larger than the peak area of donepezil obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of donepezil, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 2.0%.

Water <*2.48*> Not more than 0.2% (0.2 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Donepezil Hydrochloride and Donepezil Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Donepezil Hydrochloride), dissolve them in the mobile phase to make exactly 25 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of donepezil in each solution.

Amount (mg) of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Donepezil Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 271 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.5 g of sodium 1-decansulfonate in 650 mL of water, and add 350 mL of acetonitrile and 1 mL of perchloric acid.

Flow rate: Adjust so that the retention time of donepezil is about 11 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Donepezil Hydrochloride Fine Granules

ドネペジル塩酸塩細粒

Donepezil Hydrochloride Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl: 415.95).

Method of preparation Prepare as directed under Granules, with Donepezil Hydrochloride.

Identification To 2.5 mL of the sample solution obtained in the Assay add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 228 nm and 232 nm, between 269 nm and 273 nm, and between 313 nm and 317 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: the Donepezil Hydrochloride Fine Granules in single-dose packages meet the requirement of the Content uniformity test.

To the total amount of the content of 1 package of Donepezil Hydrochloride Fine Granule add exactly V mL of 0.1 mol/L hydrochloric acid TS so that each mL contains about 0.2 mg of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl), disperse the particles with the aid of ultrasonic waves with occasional shaking, and treat with ultrasonic waves for a further 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS, (separately determine the water <*2.48*> in the same manner as Donepezil Hydrochloride), dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of donepezil in each solution.

Amount (mg) of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl) $= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of Donepezil Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 4000 and

not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Donepezil Hydrochloride Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Donepezil Hydrochloride Fine Granules, equivalent to about 3 mg of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 55 mg of Donepezil Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Donepezil Hydrochloride), and dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid (3:1) to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of donepezil in each solution.

Dissolution rate (%) with respect to the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl)
$= M_S/M_T \times A_T/A_S \times 1/C \times 27/5$

M_S: Amount (mg) of Donepezil Hydrochloride RS taken, calculated on the anhydrous basis
M_T: Amount (mg) of Donepezil Hydrochloride Fine Granules taken
C: Labeled amount (mg) of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl) in 1 g

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

Mobile phase: A mixture of water, acetonitrile and perchloric acid (650:350:1).

Flow rate: Adjust so that the retention time of donepezil is about 4 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of throretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Assay Powder Donepezil Hydrochloride Fine Granules, if necessary. Weigh accurately a portion of the powder, equivalent to about 20 mg of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl), add 30 mL of 0.1 mol/L hydrochloric acid TS, disperse into the fine particles with the aid of ultrasonic waves with occasional shaking, and treat with ultrasonic waves for a further 15 minutes. Add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Donepezil Hydrochloride), and dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of donepezil in each solution.

Amount (mg) of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl)
$= M_S \times A_T/A_S \times 2/5$

M_S: Amount (mg) of Donepezil Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Donepezil Hydrochloride Tablets

ドネペジル塩酸塩錠

Donepezil Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl: 415.95).

Method of preparation Prepare as directed under Tablets, with Donepezil Hydrochloride.

Identification To 2.5 mL of the sample solution obtained in the Assay add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 228 nm and 232 nm, between 269 nm and 273 nm, and between 313 nm and 317 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Donepezil Hydrochloride Tablets add exactly V mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) so that each mL contains about 0.2 mg of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl), disperse with the aid of ultrasonic waves. Shake until the tablet is disintegrated, and treat with ultrasonic waves for a further 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately,

determine the water ⟨*2.48*⟩ in the same manner as Donepezil Hydrochloride), dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL. Pipet 5 mL of this solution, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of donepezil in each solution.

Amount (mg) of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl) $= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of Donepezil Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Donepezil Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Donepezil Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 3.3 μg of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 55 mg of Donepezil Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Donepezil Hydrochloride), and dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Further, pipet 3 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of donepezil in each solution.

Dissolution rate (%) with respect to the labeled amount of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl) $= M_S \times A_T/A_S \times V'/V \times 1/C \times 27/5$

M_S: Amount (mg) of Donepezil Hydrochloride RS taken, calculated on the anhydrous basis

C: Labeled amount (mg) of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl) in 1 tablet

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

Mobil phase: A mixture of water, acetonitrile and perchloric acid (650:350:1).

Flow rate: Adjust so that the retention time of donepezil is about 4 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Donepezil Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl), add 30 mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), disperse with the aid of ultrasonic waves, and add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Donepezil Hydrochloride RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Donepezil Hydrochloride), dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL. Pipet 10 mL of this solution, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of donepezil in each solution.

Amount (mg) of donepezil hydrochloride ($C_{24}H_{29}NO_3$.HCl) $= M_S \times A_T/A_S \times 2/5$

M_S: Amount (mg) of Donepezil Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Donepezil Hydrochloride.

System suitability—

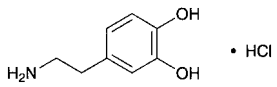
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of donepezil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of donepezil is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Dopamine Hydrochloride

ドパミン塩酸塩



$C_8H_{11}NO_2.HCl$: 189.64
4-(2-Aminoethyl)benzene-1,2-diol monohydrochloride
[*62-31-7*]

Dopamine Hydrochloride, when dried, contains not less than 98.5% of dopamine hydrochloride ($C_8H_{11}NO_2.HCl$).

Description Dopamine Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in water and in formic acid, and sparingly soluble in ethanol (95).

Melting point: about 248°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Dopamine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dopamine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dopamine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (1) for chloride.

pH <*2.54*> Dissolve 1.0 g of Dopamine Hydrochloride in 50 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Dopamine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Perform the test with 0.8 g of Dopamine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Dopamine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Dopamine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.1 g of Dopamine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, water and acetic acid (100) (16:8:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Dopamine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 15 minutes. After cooling, add 50 mL of acetic acid (100), and titrate <*2.50*> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 18.96 mg of $C_8H_{11}NO_2.HCl$

Containers and storage Containers—Tight containers.

Dopamine Hydrochloride Injection

ドパミン塩酸塩注射液

Dopamine Hydrochloride Injection is an aqueous injection.

It contains not less than 97.0% and not more than 103.0% of the labeled amount of dopamine hydrochloride ($C_8H_{11}NO_2.HCl$: 189.64).

Method of preparation Prepare as directed under Injections, with Dopamine Hydrochloride.

Description Dopamine Hydrochloride Injection occurs as a clear, colorless liquid.

Identification To a volume of Dopamine Hydrochloride Injection, equivalent to 0.04 g of Dopamine Hydrochloride, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 5 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 278 nm and 282 nm.

pH <*2.54*> 3.0 – 5.0

Bacterial endotoxins <*4.01*> Less than 4.2 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Dopamine Hydrochloride Injection, equivalent to about 30 mg of dopamine hydrochloride ($C_8H_{11}NO_2.HCl$), add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of dopamine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution

as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dopamine to that of the internal standard.

Amount (mg) of dopamine hydrochloride ($C_8H_{11}NO_2$.HCl) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of dopamine hydrochloride for assay taken

Internal standard solution—A solution of uracil in the mobile phase (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Disodium hydrogen phosphate-citric acid buffer solution (pH 3.0).

Flow rate: Adjust so that the retention time of dopamine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and dopamine are eluted in this order with the resolution between these peaks being not less than 10.

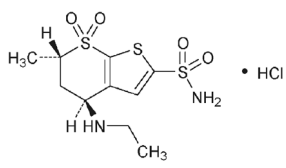
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of dopamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Dorzolamide Hydrochloride

ドルゾラミド塩酸塩



$C_{10}H_{16}N_2O_4S_3$.HCl: 360.90

(4*S*,6*S*)-4-Ethylamino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide monohydrochloride

[*130693-82-2*]

Dorzolamide Hydrochloride contains not less than 99.0% and not more than 101.0% of dorzolamide hydrochloride ($C_{10}H_{16}N_2O_4S_3$.HCl), calculated on the anhydrous basis.

Description Dorzolamide Hydrochloride occurs as a white crystalline powder.

It is soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in diluted ammonia solution (28) (13 in 400).

Optical rotation $[\alpha]_{404.7}^{25}$: -16.0 – $-17.5°$ (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Dorzolamide Hydrochloride shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Dorzolamide Hydrochloride in a solution of hydrochloric acid in methanol (9 in 1000) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dorzolamide Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dorzolamide Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Dorzolamide Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dorzolamide Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Dorzolamide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Dorzolamide Hydrochloride in 50 mL of a mixture of water and methanol (4:1), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peaks other than dorzolamide is not more than 0.1%.

Operating conditions—

Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: Adjust to pH 4.5 of a mixture of water and acetic acid (100) (1000:1) with triethylamine.

Mobile phase B: Acetonitrile.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	100	0
10 – 30	100 → 50	0 → 50

Time span of measurement: About 3 times as long as the retention time of dorzolamide, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the sample solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (4:1) to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of dorzolamide obtained with 10 μL of the solution for system suitability test is equivalent to 0.07 to 0.13% of that obtained with 10 μL of the sample solution.

System performance: To 1 mL of the sample solution add 2 mL of a mixture of water and methanol (4:1). When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dorzolamide are not less

than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 7%.

(3) Optical isomer—Dissolve 20 mg of Dorzolamide Hydrochloride in 4 mL of diluted ammonia solution (28) (13 in 400), and extract this solution with two 4-mL portions of ethyl acetate. Combine the extracts, and evaporate the ethyl acetate at 50°C under a current of nitrogen. Dissolve the residue in 3 mL of acetonitrile, add 3 drops of (*S*)-1-phenylethyl isocyanate, and allow to stand at 50°C for 10 minutes. Evaporate at 50°C under a current of nitrogen, dissolve the residue in 10 mL of a mixture of *tert*-butylmethyl ether, acetic acid (100) and acetonitrile (873:100:27), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of dorzolamide, A_2, and that of the optical isomer, having the relative retention time of about 1.5 to dorzolamide, A_1, by the automatic integration method: the result of $A_1/(A_1 + A_2)$ is not more than 0.005.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To a mixture of 30 mL of acetonitrile and 3 mL of water add *tert*-butylmethyl ether to make 1000 mL. To 650 mL of this solution add 350 mL of heptane.

Flow rate: Adjust so that the retention time of dorzolamide is about 8 minutes.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add a mixture of *tert*-butylmethyl ether, acetic acid (100) and acetonitrile (873:100:27) to make exactly 200 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of dorzolamide obtained with 5 μL of the solution for system suitability test is equivalent to 0.4 to 0.6% of that obtained with 5 μL of the sample solution.

System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dorzolamide are not less than 4000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 7%.

Water <*2.48*> Not more than 0.5% (0.5 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Dorzolamide Hydrochloride and Dorzolamide Hydrochloride RS (separately, determine the water <*2.48*> in the same manner as Dorzolamide Hydrochloride), dissolve in a mixture of water and methanol (4:1) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of dorzolamide in each solution.

Amount (mg) of dorzolamide hydrochloride ($C_{10}H_{16}N_2O_4S_3$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Dorzolamide Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8.3 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust to pH 4.5 of a mixture of water and acetic acid (100) (1000:1) with triethylamine.

Flow rate: Adjust so that the retention time of dorzolamide is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dorzolamide are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Dorzolamide Hydrochloride Ophthalmic Solution

ドルゾラミド塩酸塩点眼液

Dorzolamide Hydrochloride Ophthalmic Solution is an aqueous ophthalmic preparation.

It contains not less than 95.0% and not more than 107.0% of the labeled amount of dorzolamide ($C_{10}H_{16}N_2O_4S_3$: 324.44).

Method of preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Dorzolamide Hydrochloride.

Description Dorzolamide Hydrochloride Ophthalmic Solution occurs as a clear and colorless liquid.

Identification To a volume of Dorzolamide Hydrochloride Ophthalmic Solution, equivalent to about 1.2 mg of dorzolamide ($C_{10}H_{16}N_2O_4S_3$), add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 252 nm and 256 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Purity cis-Isomer—Use the sample solution obtained in the Assay as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and deter-

mine the peak area of dorzolamide, A_2, and that of cis-isomer, having the relative retention time of about 1.1 to dorzolamide, A_1, by the automatic integration method: $A_1/(A_1 + A_2)$ is not larger than 0.020.

Diluting solution: To 2 mL of phosphoric acid add 900 mL of water, adjust to pH 3.0 with triethylamine, then add water to make 1000 mL.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 2 mL of the sample solution add the diluting solution to make exactly 100 mL. Pipet 1 mL of this solution, add the diluting solution to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of dorzolamide obtained with 20 μL of the solution for system suitability test is equivalent to 0.07 to 0.13% of that obtained with 20 μL of the sample solution.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 7%.

Foreign insoluble matter ⟨*6.11*⟩ It meets the requirement.

Insoluble particulate matter ⟨*6.08*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Direct inoculation method, using the culture medium containing 0.7% polysorbate 80 and 0.1% of lecithin: it meets the requirement.

Assay Weigh accurately a portion of Dorzolamide Hydrochloride Ophthalmic Solution, equivalent to about 5 mg of dorzolamide ($C_{10}H_{16}N_2O_4S_3$), add the diluting solution to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Dorzolamide Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Dorzolamide Hydrochloride), dissolve in the diluting solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of dorzolamide in each solution.

Diluting solution: To 2 mL of phosphoric acid add 900 mL of water, adjust to pH 3.0 with triethylamine, then add water to make 1000 mL.

$$\text{Amount (mg/mL) of dorzolamide } (C_{10}H_{16}N_2O_4S_3) = M_S/M_T \times A_T/A_S \times 1/4 \times d \times 0.899$$

M_S: Amount (mg) of Dorzolamide Hydrochloride RS taken, calculated on the anhydrous basis

M_T: Amount (g) of Dorzolamide Hydrochloride Ophthalmic Solution taken

d: Density (g/mL) of Dorzolamide Hydrochloride Ophthalmic Solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 253 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of the diluting solution and acetonitrile (19:1).

Flow rate: Adjust so that the retention time of dorzolamide is about 10 minutes.

System suitability—

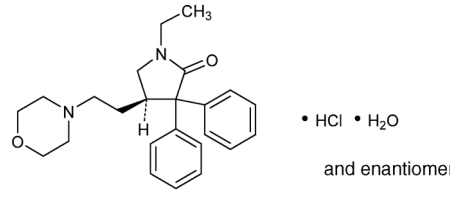
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dorzolamide are not less than 6000 and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Doxapram Hydrochloride Hydrate

ドキサプラム塩酸塩水和物



$C_{24}H_{30}N_2O_2.HCl.H_2O$: 432.98

(4*RS*)-1-Ethyl-4-[2-(morpholin-4-yl)ethyl]-3,3-diphenylpyrrolidin-2-one monohydrochloride monohydrate [*7081-53-0*]

Doxapram Hydrochloride Hydrate contains not less than 98.0% of doxapram hydrochloride ($C_{24}H_{30}N_2O_2.HCl$: 414.97), calculated on the anhydrous basis.

Description Doxapram Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in water, in ethanol (95) and in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Doxapram Hydrochloride Hydrate (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxapram Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Doxapram Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point ⟨*2.60*⟩ 218 – 222°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Perform the test with 1.0 g of Doxapram Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Doxapram Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Doxapram Hydrochloride Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.5 g of Doxapram Hydrochloride Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 6 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, formic acid, ethyl formate and methanol (8:3:3:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 3.5 – 4.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.3% (1 g).

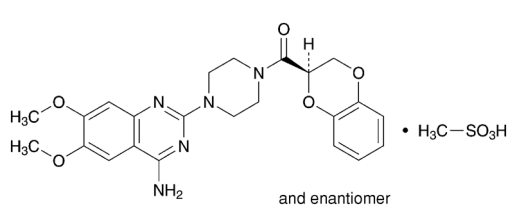
Assay Weigh accurately about 0.8 g of Doxapram Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.50 mg of $C_{24}H_{30}N_2O_2.HCl$

Containers and storage Containers—Tight containers.

Doxazosin Mesilate

ドキサゾシンメシル酸塩



and enantiomer

$C_{23}H_{25}N_5O_5.CH_4O_3S$: 547.58
1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-4-{[(2*RS*)-2,3-dihydro-1,4-benzodioxin-2-yl]carbonyl}piperazine monomethansulfonate
[*77883-43-3*]

Doxazosin Mesilate, when dried, contains not less than 98.0% and not more than 102.0% of doxazosin mesilate ($C_{23}H_{25}N_5O_5.CH_4O_3S$).

Description Doxazosin Mesilate occurs as a white to yellowish white crystalline powder.

It is freely soluble in dimethylsulfoxide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

A solution of Doxazosin Mesilate in dimethylsulfoxide solution (1 in 20) shows no optical rotation.

Melting point: about 272°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Doxazosin Mesilate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxazosin Mesilate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxazosin Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Doxazosin Mesilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) 30 mg of Doxazosin Mesilate responds to the Qualitative Tests <*1.09*> (2) for mesilate.

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of Doxazosin Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Doxazosin Mesilate in 5 mL of a mixture of methanol and acetic acid (100) (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with an upper layer of a mixture, prepared by adding 1 volume of water and 1 volume of acetic acid (100) to 2 volumes of 4-methyl-2-pentanon and shaking, to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot at the *R*f value about 0.15 obtained from the sample solution is not more intense than the spot obtained from the standard solution, and no spots other than the principal spot and other than the spots mentioned above appear from the sample solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 25 mg each of Doxazosin Mesilate and Doxazosin Mesilate RS, previously dried, dissolve separately in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of doxazosin in each solution.

Amount (mg) of doxazosin mesilate ($C_{23}H_{25}N_5O_5.CH_4O_3S$)
= $M_S \times A_T/A_S$

M_S: Amount (mg) of Doxazosin Mesilate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 246 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0), methanol and acetonitrile (12:8:3).

Flow rate: Adjust so that the retention time of doxazosin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of doxazosin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxazosin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Doxazosin Mesilate Tablets

ドキサゾシンメシル酸塩錠

Doxazosin Mesilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of doxazosin ($C_{23}H_{25}N_5O_5$: 451.48).

Method of preparation Prepare as directed under Tablets, with Doxazosin Mesilate.

Identification To a quantity of powdered Doxazosin Mesilate Tablets, equivalent to 5 mg of doxazosin ($C_{23}H_{25}N_5O_5$), add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake vigorously, and centrifuge. To 4 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid-methanol TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 244 nm and 248 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Doxazosin Mesilate Tablets add 1 mL of water, disintegrate the tablet by shaking, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL, and shake for 30 minutes. Centrifuge, pipet V mL of the supernatant liquid, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly V' mL so that each mL contains about 5 μg of doxazosin ($C_{23}H_{25}N_5O_5$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Doxazosin Mesilate RS, previously dried at 105°C for 4 hours, and dissolve in 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of doxazosin ($C_{23}H_{25}N_5O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/50 \times 0.825$

M_S: Amount (mg) of Doxazosin Mesilate RS taken

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 15 minutes of Doxazosin Mesilate Tablets is not less than 75%.

Start the test with 1 tablet of Doxazosin Mesilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add the dissolution medium to make exactly V' mL so that each mL contains about 0.56 μg of doxazosin ($C_{23}H_{25}N_5O_5$). Pipet 5 mL of this solution, add exactly 5 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Doxazosin Mesilate RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL. Then, pipet 2 mL of this solution, add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the dissolution medium, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of doxazosin in each solution.

Dissolution rate (%) with respect to the labeled amount of doxazosin ($C_{23}H_{25}N_5O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 72/25 \times 0.825$

M_S: Amount (mg) of Doxazosin Mesilate RS taken
C: Labeled amount (mg) of doxazosin ($C_{23}H_{25}N_5O_5$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 246 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 500 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10). To 450 mL of this solution add 550 mL of methanol.

Flow rate: Adjust so that the retention time of doxazosin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of doxazosin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxazosin is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Doxazosin Mesilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of doxazosin ($C_{23}H_{25}N_5O_5$), add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL, and stir for 30 minutes. Centrifuge, pipet 4 mL of the supernatant liquid, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of Doxazosin Mesilate RS, previously dried at 105°C for 4 hours, dissolve

in 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid-methanol TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 246 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

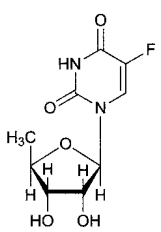
Amount (mg) of doxazosin ($C_{23}H_{25}N_5O_5$)
$= M_S \times A_T/A_S \times 1/4 \times 0.825$

M_S: Amount (mg) of Doxazosin Mesilate RS taken

Containers and storage Containers—Well-closed containers.

Doxifluridine

ドキシフルリジン



$C_9H_{11}FN_2O_5$: 246.19
5′-Deoxy-5-fluorouridine
[*3094-09-5*]

Doxifluridine, when dried, contains not less than 98.5% and not more than 101.0% of doxifluridine ($C_9H_{11}FN_2O_5$).

Description Doxifluridine occurs as a white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, soluble in water and in methanol, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS and in 0.01 mol/L sodium hydroxide TS.

Melting point: about 191°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Doxifluridine in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxifluridine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_{365}^{20}$: +160 – +174° (after drying, 0.1 g, water, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Doxifluridine in 10 mL of water is between 4.2 and 5.2.

Purity (1) Fluoride—Dissolve 0.10 g of Doxifluridine in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution into a 20-mL volumetric flask, add 5 mL of a mixture of acetone and lanthanum-alizarin complexone TS (2:1) and water to make 20 mL, allow to stand for 1 hour, and use this solution as the sample solution. Separately, put 1.0 mL of Standard Fluorine Solution in a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) and 5 mL of the mixture of acetone and alizarin complexone TS (2:1), then proceed in the same manner as for preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 620 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained in the same way with 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) as a blank: A_T is not larger than A_S.

(2) Chloride <1.03>—Perform the test with 0.30 g of Doxifluridine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.035%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Doxifluridine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 20 mg of Doxifluridine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 25 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100) and water (17:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot with the sample solution is not more than three, and they are not more intense than the spot with the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.25 g of Doxifluridine, previously dried, dissolve in 50 mL of *N*,*N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
$= 24.62$ mg of $C_9H_{11}FN_2O_5$

Containers and storage Containers—Tight containers.

Doxifluridine Capsules

ドキシフルリジンカプセル

Doxifluridine Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of doxifluridine ($C_9H_{11}FN_2O_5$: 246.19).

Method of preparation Prepare as directed under Capsules, with Doxifluridine.

Identification (1) Dissolve an amount of the contents of Doxifluridine Capsules, equivalent to 20 mg of Doxifluridine, in 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. To 1 mL of the filtrate add 0.1 mol/L hydrochlo-

ric acid TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using 0.1 mol/L hydrochloric acid TS as the blank: it exhibits a maximum between 267 nm and 271 nm.

(2) To an amount of powdered contents of Doxifluridine Capsules, equivalent to 20 mg of Doxifluridine, add 2 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of doxifluridine in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100) and water (17:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot with the sample solution and the spot with the standard solution show a dark purple color and these *R*f values are the same.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Doxifluridine Capsules is not less than 85%.

Start the test with 1 capsule of Doxifluridine Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 13 μg of doxifluridine ($C_9H_{11}FN_2O_5$), and use this solution as the sample solution. Separately, weigh accurately about 26 mg of doxifluridine for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of doxifluridine ($C_9H_{11}FN_2O_5$)
$= M_S \times A_T/A_S \times V/V' \times 1/C \times 45$

M_S: Amount (mg) of doxifluridine for assay taken
C: Labeled amount (mg) of doxifluridine ($C_9H_{11}FN_2O_5$) in 1 capsule

Assay Weigh accurately the mass and powder the contents of not less than 20 Doxifluridine Capsules. Weigh accurately a portion of the powder, equivalent to about 50 mg of doxifluridine ($C_9H_{11}FN_2O_5$), add 40 mL of water, shake for 10 minutes, add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, then add a mixture of water and methanol (5:3) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of doxifluridine for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mixture of water and methanol (5:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of doxifluridine to that of the internal standard.

Amount (mg) of doxifluridine ($C_9H_{11}FN_2O_5$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of doxifluridine for assay taken

Internal standard solution—A solution of anhydrous caffeine (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (13:7).

Flow rate: Adjust so that the retention time of doxifluridine is about 2.5 minutes.

System suitability—

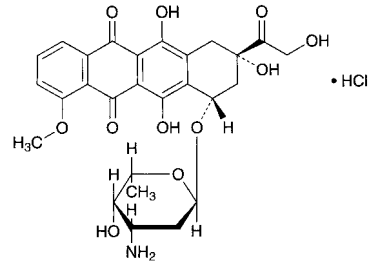
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, doxifluridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of doxifluridine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Doxorubicin Hydrochloride

ドキソルビシン塩酸塩



$C_{27}H_{29}NO_{11}$.HCl: 579.98
(2*S*,4*S*)-4-(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyloxy)-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride
[*25316-40-9*]

Doxorubicin Hydrochloride is the hydrochloride of a derivative of daunorubicin.

It contains not less than 980 μg (potency) and not more than 1080 μg (potency) per mg, calculated on the anhydrous basis. The potency of Doxorubicin Hydrochloride is expressed as mass (potency) of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl).

Description Doxorubicin Hydrochloride occurs as a red-orange crystalline powder.

It is sparingly soluble in water, slightly soluble in metha-

nol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification **(1)** Determine the absorption spectrum of a solution of Doxorubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxorubicin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxorubicin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Doxorubicin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Doxorubicin Hydrochloride (1 in 200) responds to the Qualitative Tests <1.09> (1) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +240 – +290° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 50 mg of Doxorubicin Hydrochloride in 10 mL of water is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 50 mg of Doxorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Related substances—Dissolve 25 mg of Doxorubicin Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than doxorubicin obtained from the sample solution is not larger than 1/4 times the peak area of doxorubicin obtained from the standard solution, and the total area of the peaks other than doxorubicin is not larger than the peak area of doxorubicin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000), and add 1000 mL of acetonitrile.

Flow rate: Adjust so that the retention time of doxorubicin is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of doxorubicin.

System suitability—

Test for required detectability: Measure 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of doxorubicin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Doxorubicin Hydrochloride in 20 mL of water, add 1.5 mL of phosphoric acid, and allow to stand at room temperature for 30 minutes. Adjust the pH of this solution to 2.5 with 2 mol/L sodium hydroxide TS. When the procedure is run with 20 μL of this solution under the above operating conditions, doxorubicinone, having the relative retention time of about 0.6 to doxorubicin, and doxorubicin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxorubicin is not more than 2.0%.

Water <2.48> Not more than 3.0% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Doxorubicin Hydrochloride and Doxorubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in water to make exactly 25 mL. Pipet 5 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances at 495 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount [μg (potency)] of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl)
$= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Doxorubicin Hydrochloride RS taken

Containers and storage Containers—Tight containers.

Doxorubicin Hydrochloride for Injection

注射用ドキソルビシン塩酸塩

Doxorubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl: 579.98).

Method of preparation Prepare as directed under Injections, with Doxorubicin Hydrochloride.

Description Doxorubicin Hydrochloride for Injection occurs as red-orange, powder or masses.

Identification Dissolve an amount of Doxorubicin Hydrochloride for Injection, equivalent to 10 mg (potency) of Doxorubicin Hydrochloride, in methanol to make 100 mL. To 5 mL of this solution add methanol to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 231 nm and 235 nm, between 250 nm and 254 nm, between 477 nm and 481 nm, and between 493 nm and 497 nm, and exhibits a shoulder between 528 nm and 538 nm.

pH <2.54> The pH of a solution, prepared by dissolving an amount of Doxorubicin Hydrochloride for Injection equivalent to 10 mg (potency) of Doxorubicin Hydrochloride, in 2 mL of water, is 5.0 to 6.0.

Purity Clarity and color of solution—Dissolve an amount of Doxorubicin Hydrochloride for Injection, equivalent to 50 mg (potency) of Doxorubicin Hydrochloride, in 10 mL of water: the solution is clear and red.

Water ⟨*2.48*⟩ Not more than 4.0% (0.25 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.50 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Doxorubicin Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Doxorubicin Hydrochloride, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use the solution as the sample solution. Separately, weigh accurately an amount of Doxorubicin Hydrochloride RS, equivalent to 10 mg (potency), add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and calculate the ratios, Q_T and Q_S, of the peak area of doxorubicin to that of the internal standard.

Amount [mg (potency)] of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl)
$= M_S \times Q_T/Q_S$

M_S: Amount [mg (potency)] of Doxorubicin Hydrochloride RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000). To this solution add 1000 mL of acetonitrile.

Flow rate: Adjust so that the retention time of doxorubicin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, doxorubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5, and the symmetry factor of the peak of doxorubicin is between 0.8 and 1.2.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of doxorubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Doxycycline Hydrochloride Hydrate

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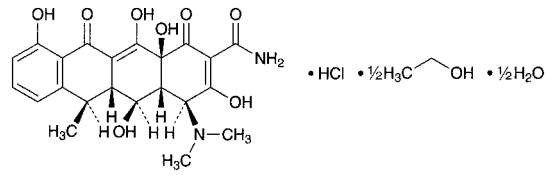

$C_{22}H_{24}N_2O_8$.HCl.½C_2H_6O.½H_2O: 512.94

(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-Dimethylamino-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride hemiethanolate hemihydrate

[*564-25-0*, Doxycycline]

Doxycycline Hydrochloride Hydrate is the hydrochloride of a derivative of oxytetracycline.

It contains not less than 880 μg (potency) and not more than 943 μg (potency) per mg, calculated on the anhydrous and residual ethanol-free basis. The potency of Doxycycline Hydrochloride Hydrate is expressed as mass (potency) of doxycycline ($C_{22}H_{24}N_2O_8$: 444.43).

Description Doxycycline Hydrochloride Hydrate occurs as yellow to dark yellow, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification **(1)** Determine the infrared absorption spectrum of Doxycycline Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Doxycycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg of Doxycycline Hydrochloride Hydrate in 10 mL of water, and add silver nitrate TS: a white turbidity is produced.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (349 nm): 285 – 315 (10 mg, 0.01 mol/L hydrochloric acid-methanol TS, 500 mL).

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −105 – −120° (0.25 g calculated on the anhydrous and residual ethanol-free basis, 0.01 mol/L hydrochloric acid-methanol TS, 25 mL, 100 mm). Determine within 5 minutes after the sample solution is prepared.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Doxycycline Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 5.0 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substance—Dissolve 20 mg of Doxycycline Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 6-epidoxycycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 6-epidoxycycline hydrochloride stock solution. Separately, dissolve 20 mg of metacycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as metacycline hydrochloride stock solution. Pipet 2 mL each of 6-epidoxycycline hydrochloride stock solution and metacycline

hydrochloride stock solution, add 0.01 mol/L hydrochloric acid TS to make exatly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of metacycline and 6-epidoxycycline obtained from the sample solution are not larger than the peak areas of them obtained from the standard solution, respectively, and the areas of the peaks, appeared between the solvent peak and metacycline and behind of doxycycline, from the sample solution are not larger than 1/4 times the peak area of 6-epidoxycycline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Mix 125 mL of 0.2 mol/L potassium dihydrogen phosphate TS, 117 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 500 mL. To 400 mL of this solution add 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 25), 60 g of *t*-butanol and 200 mL of water, adjust to pH 8.0 with 2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of doxycycline is about 19 minutes.

Time span of measurement: About 2.4 times as long as the retention time of doxycycline, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak areas of 6-epidoxycycline and metacycline obtained from 20 μL of this solution are equivalent to 3.5 to 6.5% of them obtained from 20 μL of the standard solution, respectively.

System performance: To 8 mL of the sample solution, 3 mL of 6-epidoxycycline hydrochloride stock solution and 2 mL of metacycline hydrochloride stock solution add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, metacycline, 6-epidoxycycline and doxycycline are eluted in this order with the resolutions between the peaks, metacycline and 6-epidoxycycline, and 6-epidoxycycline and doxycycline, being not less than 1.3 and not less than 2.0, respectively, and the symmetry factor of the peak of doxycycline is not more than 1.3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of metacycline and 6-epidoxycycline are not more than 3.0% and not more than 2.0%, respectively.

Ethanol Weigh accurately about 0.1 g of Doxycycline Hydrochloride Hydrate, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of ethanol (99.5), and add the internal standard solution to make exactly 100 mL. Pipet 1 mL of this solution, add the internal standard solution to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethanol to that of the internal standard: the amount of ethanol is not less than 4.3% and not more than 6.0%.

$$\text{Amount (\%) of ethanol} = M_S/M_T \times Q_T/Q_S$$

M_S: Amount (mg) of ethanol (99.5) taken

M_T: Amount (mg) of Doxycycline Hydrochloride Hydrate taken

Internal standard solution—A solution of 1-propanol (1 in 2000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3.2 mm in inside diameter and 1.5 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (0.0075 μm in average pore size, 500 – 600 m^2/g in specific surface area) (150 – 180 μm in particle diameter).

Column temperature: A constant temperature of about 135°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethanol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water ⟨*2.48*⟩ Not less than 1.4% and not more than 2.8% (0.6 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.3% (1 g).

Assay Weigh accurately an amount of Doxycycline Hydrochloride Hydrate and Doxycycline Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in water to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of doxycycline in each solution.

$$\text{Amount [}\mu\text{g (potency)] of doxycycline } (C_{22}H_{24}N_2O_8) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Doxycycline Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 7.0 g of sodium dihydrogen phos-

phate dihydrate in 450 mL of water, add 553 mL of a mixture of methanol and *N*,*N*-dimethyl-*n*-octylamine (550:3), and adjust the pH to 8.0 with a solution of sodium hydroxide (43 in 200).

Flow rate: Adjust so that the retention time of doxycycline is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetry factor of the peak of doxycycline are not less than 1000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxycycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Doxycycline Hydrochloride Tablets

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Doxycycline Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled potency of doxycycline ($C_{22}H_{24}N_2O_8$: 444.43).

Method of preparation Prepare as directed under Tablets, with Doxycycline Hydrochloride Hydrate.

Identification Weigh a portion of powdered Doxycycline Hydrochloride Tablets, equivalent to 1 mg (potency) of Doxycycline Hydrochloride Hydrate, add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake thoroughly, and filter. Determine the absorption spectrum of this filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 266 nm and 271 nm and between 347 nm and 353 nm.

Purity 4-Epidoxycycline—Use the sample solution obtained in the Assay as the sample solution. Pipet 2 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 0.6 to doxycycline, obtained from the sample solution is not larger than 1.5 times the peak area of doxycycline obtained from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of doxycycline obtained with 10 μL of this solution is equivalent to 7 to 13% of the peak area of doxycycline obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of doxycycline are not less than 2200 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of doxycycline is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Doxycycline Hydrochloride Tablets add 0.01 mol/L hydrochloric acid TS, disperse the tablet with the aid of ultrasonic waves, shake for 15 minutes, then add 0.01 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg (potency) of Doxycycline Hydrochloride Hydrate. Centrifuge this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of doxycycline ($C_{22}H_{24}N_2O_8$)
$= M_S \times A_T/A_S \times V/20$

M_S: Amount [mg (potency)] of Doxycycline Hydrochloride RS taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Doxycycline Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Doxycycline Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg (potency) of Doxycycline Hydrochloride Hydrate, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Doxycycline Hydrochloride RS, dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 274 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount [mg (potency)] of Doxycycline Hydrochloride RS taken

C: Labeled amount [mg(potency)] of doxycycline ($C_{22}H_{24}N_2O_8$) in 1 tablet

Assay To 10 Doxycycline Hydrochloride Tablets add 0.01 mol/L hydrochloric acid TS, disperse them with the aid of ultrasonic waves, shake for 15 minutes, and add 0.01 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 2 mg (potency) of Doxycycline Hydrochloride Hydrate. Centrifuge, if necessary, pipet 10 mL of the supernatant liquid, add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Doxycycline Hydrochloride RS, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard

solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of doxycycline in each solution.

Amount [mg (potency)] of doxycycline ($C_{22}H_{24}N_2O_8$) in 1 tablet
$= M_S \times A_T/A_S \times V/100$

M_S: Amount [mg (potency)] of Doxycycline Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 7.0 g of sodium dihydrogen phosphate dihydrate in 450 mL of water. Add to this solution 553 mL of a mixture of methanol and *N*,*N*-dimethyl-*n*-octylamine (550:3), and adjust to pH 8.0 with sodium hydroxide solution (43 in 200).

Flow rate: Adjust so that the retention time of doxycycline is about 6 minutes.

System suitability—

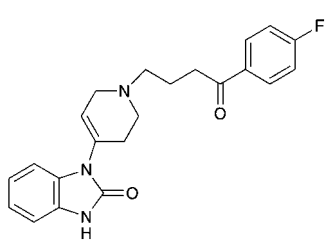
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of doxycycline are not less than 2200 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxycycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Droperidol

ドロペリドール



$C_{22}H_{22}FN_3O_2$: 379.43
1-{1-[4-(4-Fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridin-4-yl}-1,3-dihydro-2*H*-benzimidazol-2-one
[*548-73-2*]

Droperidol, when dried, contains not less than 98.0% of droperidol ($C_{22}H_{22}FN_3O_2$).

Description Droperidol occurs as a white to light yellow powder.

It is freely soluble in acetic acid (100), soluble in dichloromethane, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually colored by light.

It shows crystal polymorphism.

Identification **(1)** Put 30 mg of Droperidol in a brown volumetric flask, and dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Transfer 5 mL of the solution to a brown volumetric flask, and add 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Droperidol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Droperidol in acetone, evaporate the acetone, dry the residue in a desiccator (in vacuum, silica gel, 70°C) for 4 hours, and perform the test with the residue.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Droperidol in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 50 mg of Droperidol in 5 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, chloroform, methanol and acetic acid-sodium acetate buffer solution (pH 4.7) (54:23:18:5) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 3.0% (0.5 g, in vacuum, silica gel, 70°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g, platinum crucible).

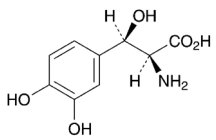
Assay Weigh accurately about 0.5 g of Droperidol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
$= 37.94$ mg of $C_{22}H_{22}FN_3O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Droxidopa

ドロキシドパ



$C_9H_{11}NO_5$: 213.19
(2*S*,3*R*)-2-Amino-3-(3,4-dihydroxyphenyl)-3-hydroxypropanoic acid
[*23651-95-8*]

Droxidopa, when dried, contains not less than 99.0% and not more than 101.0% of droxidopa ($C_9H_{11}NO_5$).

Description Droxidopa occurs as white to light brown, crystals or crystalline powder.

It is slightly soluble in water and practically insoluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification **(1)** Determine the absorption spectrum of a solution of Droxidopa in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Droxidopa as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -38 – $-43°$ (after drying, 0.1 g, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

Purity **(1)** Chloride <*1.03*>—Dissolve 0.40 g of Droxidopa in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Droxidopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Droxidopa according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—To 0.10 g of Droxidopa add 50 mL of 0.1 mol/L hydrochloric acid TS, dissolve by shaking while cooling in an ice bath, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than droxidopa obtained from the sample solution is not larger than the peak area of droxidopa obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate and 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.0 with phosphoric acid. To 930 mL of this solution add 70 mL of acetonitrile.

Flow rate: Adjust so that the retention time of droxidopa is about 5 minutes.

Time span of measurement: About 12 times as long as the retention time of droxidopa, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of droxidopa are not less than 10,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of droxidopa is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.1% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Droxidopa, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS, add 50 mL of acetic acid (100), and titrate <*2.50*> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.32 mg of $C_9H_{11}NO_5$

Containers and storage Containers—Well-closed containers.

Droxidopa Capsules

ドロキシドパカプセル

Droxidopa Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of droxidopa ($C_9H_{11}NO_5$: 213.19).

Method of preparation Prepare as directed under Capsules, with Droxidopa.

Identification **(1)** To an amount of the contents of Droxidopa Capsules, equivalent to 50 mg of Droxidopa, add 50 mL of water, shake for 10 minutes, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat in a water bath for 3 minutes: a blue-purple color develops.

(2) To an amount of the contents of Droxidopa Capsules, equivalent to 20 mg of Droxidopa, add 20 mL of diluted acetic acid (100) (1 in 500), shake for 10 minutes, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to light brown.

(3) To an amount of the contents of Droxidopa Capsules, equivalent to 50 mg of Droxidopa, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hy-

drochloric acid TS to make 100 mL, and filter. Discard the first 10 mL of the filtrate, and to 2 mL of the subsequent filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 278 nm and 282 nm.

Uniformity of dosage units <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To the contents of 1 capsule of Droxidopa Capsules, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake well, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.5 mg of droxidopa ($C_9H_{11}NO_5$). Filter this solution, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 280 nm.

Amount (mg) of droxidopa ($C_9H_{11}NO_5$)
$= M_S \times A_T/A_S \times V/100$

M_S: Amount (mg) of droxidopa for assay taken

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Droxidopa Capsules is not less than 70%.

Start the test with 1 capsule of Droxidopa Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of droxidopa ($C_9H_{11}NO_5$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_{T1} and A_{S1}, at 280 nm, and A_{T2} and A_{S2}, at 350 nm.

Dissolution rate (%) with respect to the labeled amount of droxidopa ($C_9H_{11}NO_5$)
$= M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of droxidopa for assay taken
C: Labeled amount (mg) of droxidopa ($C_9H_{11}NO_5$) in 1 capsule

Assay Take out the contents of not less than 20 Droxidopa Capsules, weigh accurately the mass of the contents, and mix uniformly. Weigh accurately an amount equivalent to about 50 mg of droxidopa ($C_9H_{11}NO_5$), add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 280 nm.

Amount (mg) of droxidopa ($C_9H_{11}NO_5$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of droxidopa for assay taken

Containers and storage Containers—Tight containers.

Droxidopa Fine Granules

ドロキシドパ細粒

Droxidopa Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of droxidopa ($C_9H_{11}NO_5$: 213.19).

Method of preparation Prepare as directed under Granules, with Droxidopa.

Identification **(1)** To a quantity of powdered Droxidopa Fine Granules, equivalent to 50 mg of Droxidopa, add 50 mL of water, shake for 10 minutes, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, heat in a water bath for 3 minutes: a blue-purple color develops.

(2) To a quantity of powdered Droxidopa Fine Granules, equivalent to 20 mg of Droxidopa, add 20 mL of diluted acetic acid (100) (1 in 500), shake for 10 minutes, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to light brown.

(3) To a quantity of powdered Droxidopa Fine Granules, equivalent to 50 mg of Droxidopa, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. Discard the first 10 mL of the filtrate, to 2 mL of the subsequent filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 278 nm and 282 nm.

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Droxidopa Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Droxidopa Fine Granules, equivalent to about 0.1 g of droxidopa ($C_9H_{11}NO_5$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of water, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution.

Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_{T1} and A_{S1}, at 280 nm, and A_{T2} and A_{S2}, at 350 nm.

Dissolution rate (%) with respect to the labeled amount of droxidopa ($C_9H_{11}NO_5$)
$= M_S/M_T \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times 1/C \times 360$

M_S: Amount (mg) of droxidopa for assay taken
M_T: Amount (g) of Droxidopa Fine Granules taken
C: Labeled amount (mg) of droxidopa ($C_9H_{11}NO_5$) in 1 g

Assay Powder not less than 20 g of Droxidopa Fine Granules. Weigh accurately a portion of the powder, equivalent to about 50 mg of droxidopa ($C_9H_{11}NO_5$), add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 280 nm.

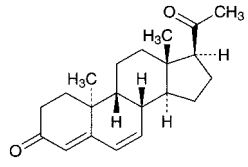
Amount (mg) of droxidopa ($C_9H_{11}NO_5$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of droxidopa for assay taken

Containers and storage Containers—Tight containers.

Dydrogesterone

ジドロゲステロン



$C_{21}H_{28}O_2$: 312.45
9β,10α-Pregna-4,6-diene-3,20-dione
[*152-62-5*]

Dydrogesterone, when dried, contains not less than 98.0% and not more than 102.0% of dydrogesterone ($C_{21}H_{28}O_2$).

Description Dydrogesterone occurs as white to light yellowish white, crystals or crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetonitrile, sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification **(1)** To 5 mg of Dydrogesterone add 5 mL of 4-methoxybenzaldehyde-acetic acid TS and 2 to 3 drops of sulfuric acid, and heat in a water bath for 2 minutes: an orange-red color develops.

(2) Determine the absorption spectrum of a solution of Dydrogesterone in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Dydrogesterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -470 – $-500°$ (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 167 – 171°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Dydrogesterone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 10 mg of Dydrogesterone in 200 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than dydrogesterone from the sample solution is not larger than the peak area of dydrogesterone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, ethanol (95) and acetonitrile (53:26:21).

Flow rate: Adjust so that the retention time of dydrogesterone is about 12 minutes.

Selection of column: Dissolve 1 mg each of Dydrogesterone and progesterone in 20 mL of the mobile phase. Proceed with 10 μL of the solution under the above operating conditions, and calculate the resolution. Use a column giving elution of dydrogesterone and progesterone in this order with the resolution between these peaks being not less than 8. Wavelength is 265 nm.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dydrogesterone obtained from 10 μL of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of dydrogesterone, beginning after the solvent peak.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg of Dydrogesterone, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 1 mL of this solution, and add methanol to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 286 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of dydrogesterone ($C_{21}H_{28}O_2$)
$= A/845 \times 100{,}000$

Containers and storage Containers—Tight containers.

Dydrogesterone Tablets

ジドロゲステロン錠

Dydrogesterone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of dydrogesterone ($C_{21}H_{28}O_2$: 312.45).

Method of preparation Prepare as directed under Tablets, with Dydrogesterone.

Identification (1) To a quantity of powdered Dydrogesterone Tablets, equivalent to 0.05 g of Dydrogesterone, add 50 mL of methanol, shake well, and filter. Evaporate 5 mL of the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Dydrogesterone.

(2) To 1 mL of the filtrate obtained in (1) add methanol to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 284 nm and 288 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Crush 1 tablet of Dydrogesterone Tablets, and add methanol to make exactly 100 mL. Shake until the tablet is completely disintegrated, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V' mL so that each mL contains about 5 μg of dydrogesterone ($C_{21}H_{28}O_2$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of dydrogesterone ($C_{21}H_{28}O_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/20$

M_S: Amount (mg) of dydrogesterone for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Dydrogesterone Tablets is not less than 80%.

Start the test with 1 tablet of Dydrogesterone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of dydrogesterone ($C_{21}H_{28}O_2$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dydrogesterone for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 296 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control.

Dissolution rate (%) with respect to the labeled amount of dydrogesterone ($C_{21}H_{28}O_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of dydrogesterone for assay taken
C: Labeled amount (mg) of dydrogesterone ($C_{21}H_{28}O_2$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Dydrogesterone Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of dydrogesterone ($C_{21}H_{28}O_2$), shake with 50 mL of methanol, and add methanol to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dydrogesterone for assay, previously dried in vacuum for 24 hours using phosphorus (V) oxide as a dessicant, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use the solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 286 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

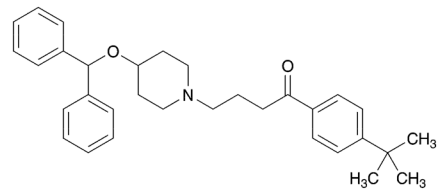
Amount (mg) of dydrogesterone ($C_{21}H_{28}O_2$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of dydrogesterone for assay taken

Containers and storage Containers—Tight containers.

Ebastine

エバスチン



$C_{32}H_{39}NO_2$: 469.66
1-[4-(1,1-Dimethylethyl)phenyl]-
4-[4-(diphenylmethoxy)piperidin-
1-yl]butan-1-one
[*90729-43-4*]

Ebastine, when dried, contains not less than 99.0% and not more than 101.0% of ebastine ($C_{32}H_{39}NO_2$).

Description Ebastine occurs as white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

It gradually becomes yellowish white on exposure to light.

Identification (1) Dissolve 20 mg of Ebastine in 5 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of sodium hydroxide TS, and allow to stand: the color of the solution is purple to red-purple, which gradually changes to blown.

(2) Determine the absorption spectrum of a solution of Ebastine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit

similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ebastine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 84 – 87°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ebastine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm). A platinum crucible may be used.

(2) Related substances—Dissolve 0.10 g of Ebastine in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exacty 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than ebastine obtained from the sample solution is not larger than the peak area of ebastine obtained from the standard solution, and the total area of the peaks other than ebastine from the sample solution is not larger than 4 times the peak area of ebastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 5), and add water to make 1000 mL. To 375 mL of this solution add 625 mL of acetonitrile for liquid chromatography, and dissolve 0.72 g of sodium lauryl sulfate in this solution.

Flow rate: Adjust so that the retention time of ebastine is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of ebastine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ebastine obtained with 10 μL of this solution is equivalent to 35 to 65% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ebastine are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ebastine is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ebastine, previously dried, dissolve in 60 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.97 mg of $C_{32}H_{39}NO_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Ebastine Orally Disintegrating Tablets

エバスチン口腔内崩壊錠

Ebastine Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ebastine ($C_{32}H_{39}NO_2$: 469.66).

Method of preparation Prepare as directed under Tablets, with Ebastine.

Identification Powder Ebastine Orally Disintegrating Tablets. To a potion of the powder, equivalent to 30 mg of Ebastine, add 70 mL of methanol, shake for 10 minutes, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 251 nm and 255 nm.

Purity Related substances—Powder Ebastine Orally Disintegrating Tablets. To a portion of the powder, equivalent to 50 mg of Ebastine, add 30 mL of methanol for liquid chromatography, shake for 10 minutes, and add the mobile phase to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ebastine obtained from the sample solution is not larger than the peak area of ebastine obtained from the standard solution, and the total area of the peaks other than ebastine from the sample solution is not larger than 2 times the peak area of ebastine from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 3 times as long as the retention time of ebastine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of ebastine obtained with 10 μL of this solution is equivalent to 15 to 25% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of ebastine are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ebastine is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ebastine Orally Disintegrating Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves with occasional shaking. Add 3V/5 mL of methanol, shake for 10 minutes, then add methanol to make exactly V mL so that each mL contains about 0.1 mg of ebastine ($C_{32}H_{39}NO_2$), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of ebastine } (C_{32}H_{39}NO_2) = M_S \times Q_T/Q_S \times V/500$$

M_S: Amount (mg) of ebastine for assay taken

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 40,000).

Disintegration Being specified separately when the drug is granted approval based on the Law.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Ebastine Orally Disintegrating Tablets is not less than 80%.

Start the test with 1 tablet of Ebastine Orally Disintegrating Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of ebastine ($C_{32}H_{39}NO_2$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of ebastine } (C_{32}H_{39}NO_2) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of ebastine for assay taken
C: Labeled amount (mg) of ebastine ($C_{32}H_{39}NO_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Ebastine Orally Disintegrating Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of ebastine ($C_{32}H_{39}NO_2$), add 20 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves. Add 120 mL of methanol, shake for 10 minutes, add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of 0.1 mol/L hydrochloric acid TS, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ebastine to that of the internal standard.

$$\text{Amount (mg) of ebastine } (C_{32}H_{39}NO_2) = M_S \times Q_T/Q_S \times 2/5$$

M_S: Amount (mg) of ebastine for assay taken

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 5), and add water to make 1000 mL. To 375 mL of this solution add 625 mL of acetonitrile for liquid chromatography, and dissolve 0.72 g of sodium lauryl sulfate in this solution.

Flow rate: Adjust so that the retention time of ebastine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ebastine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ebastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ebastine Tablets

エバスチン錠

Ebastine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ebastine ($C_{32}H_{39}NO_2$: 469.66).

Method of preparation Prepare as directed under Tablets, with Ebastine.

Identification Powder Ebastine Tablets. To a potion of the powder, equivalent to 30 mg of Ebastine, add 70 mL of methanol, shake for 10 minutes, then add methanol to make

100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 251 nm and 255 nm.

Purity Related substances—Powder Ebastine Tablets. To a portion of the powder, equivalent to 50 mg of Ebastine, add 30 mL of methanol for liquid chromatography, shake for 10 minutes, and add the mobile phase to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ebastine obtained from the sample solution is not larger than the peak area of ebastine obtained from the standard solution, and the total area of the peaks other than ebastine from the sample solution is not larger than 2 times the peak area of ebastine from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 3 times as long as the retention time of ebastine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of ebastine obtained with 10 μL of this solution is equivalent to 15 to 25% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ebastine are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ebastine is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ebastine Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves with occasional shaking. Add $3V$/5 mL of methanol, shake for 10 minutes, then add methanol to make exactly V mL so that each mL contains about 0.1 mg of ebastine ($C_{32}H_{39}NO_2$), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of ebastine } (C_{32}H_{39}NO_2) = M_S \times Q_T/Q_S \times V/500$$

M_S: Amount (mg) of ebastine for assay taken

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 40,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Ebastine Tablets is not less than 75%.

Start the test with 1 tablet of Ebastine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of ebastine ($C_{32}H_{39}NO_2$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of ebastine } (C_{32}H_{39}NO_2) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of ebastine for assay taken

C: Labeled amount (mg) of ebastine ($C_{32}H_{39}NO_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Ebastine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of ebastine ($C_{32}H_{39}NO_2$), add 20 mL of 0.1 mol/L hydrochloric acid TS, and disperse the particles with the aid of ultrasonic waves. Add 120 mL of methanol, shake for 10 minutes, add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ebastine for assay, previously dried at 60°C under reduced pressure with phosphorous (V) oxide for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of 0.1 mol/L hydrochloric acid TS, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ebastine to that of the internal standard.

$$\text{Amount (mg) of ebastine } (C_{32}H_{39}NO_2) = M_S \times Q_T/Q_S \times 2/5$$

M_S: Amount (mg) of ebastine for assay taken

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 5), and add water to make 1000

mL. To 375 mL of this solution add 625 mL of acetonitrile for liquid chromatography, and dissolve 0.72 g of sodium lauryl sulfate in this solution.

Flow rate: Adjust so that the retention time of ebastine is about 9 minutes.

System suitability—

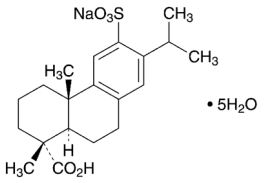
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ebastine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ebastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ecabet Sodium Hydrate

エカベトナトリウム水和物



$C_{20}H_{27}NaO_5S.5H_2O$: 492.56

(1*R*,4a*S*,10a*S*)-1,4a-Dimethyl-7-(1-methylethyl)-6-sodiosulfonato-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid pentahydrate

[*219773-47-4*]

Ecabet Sodium Hydrate contains not less than 98.5% and not more than 101.5% of ecabet sodium ($C_{20}H_{27}NaO_5S$: 402.48), calculated on the anhydrous basis.

Description Ecabet Sodium Hydrate is white crystals.

It is freely soluble in methanol, and slightly soluble in water and in ethanol (99.5).

It dissolves in sodium hydroxide TS.

Dissolve 1.0 g of Ecabet Sodium Hydrate in 200 mL of water: the pH of the solution is about 3.5.

Identification (1) Determine the absorption spectrum of a solution of Ecabet Sodium Hydrate in dilute sodium hydroxide TS (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ecabet Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Place 1 g of Ecabet Sodium Hydrate in a porcelain crucible, and carbonize. After cooling, add 0.5 mL of nitric acid, heat gradually to incinerate, and dissolve the residue in 10 mL of water: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +69 – +76° (0.25 g calculated on the anhydrous basis, methanol, 25 mL, 100 mm).

Purity (1) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ecabet Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 10 mg of Ecabet Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the area of each peak other than ecabet obtained from the sample solution is not larger than the peak area of ecabet obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.1 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 3.0. To 730 mL of this solution add 270 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ecabet is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of ecabet, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ecabet are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ecabet is not more than 2.0%.

Water ⟨*2.48*⟩ 17.3 – 19.2% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 1.2 g of Ecabet Sodium Hydrate, dissolve in 30 mL of methanol, add 30 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 4 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{20}H_{27}NaO_5S$

Containers and storage Containers—Well-closed containers.

Ecabet Sodium Granules

エカベトナトリウム顆粒

Ecabet Sodium Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S.5H_2O$: 492.56).

Method of preparation Prepare as directed under Granules, with Ecabet Sodium Hydrate.

Identification To a quantity of Ecabet Sodium Granules, equivalent to 50 mg of Ecabet Sodium Hydrate, add 25 mL of dilute sodium hydroxide TS, shake, and filter. Discard the first 10 mL of the filtrate, and to 3 mL of the subsequent filtrate add dilute sodium hydroxide TS to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 269 nm and 273 nm, and between 278 nm and 282 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Ecabet Sodium Granules in single-dose packages meet the requirement of the Content uniformity test.

Take out the total amount of the content of 1 package of Ecabet Sodium Granules, add 70 mL of dilute sodium hydroxide TS, treat with ultrasonic waves for 5 minutes with occasional shaking, add dilute sodium hydroxide TS to make exactly V mL so that each mL contains about 10 mg of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S.5H_2O$), and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of ecabet sodium hydrate for assay (separately, determine the water ⟨*2.48*⟩ in the same manner as Ecabet Sodium Hydrate), dissolve in 2 mL of dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 271 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank.

Amount (mg) of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S.5H_2O$)
$= M_S \times A_T/A_S \times V/2 \times 1.224$

M_S: Amount (mg) of ecabet sodium hydrate for assay taken, calculated on the anhydrous basis

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ecabet Sodium Granules is not less than 80%.

Start the test with an accurately weighed amount of Ecabet Sodium Granules, equivalent to about 1 g of Ecabet Sodium Hydrate, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of ecabet sodium hydrate for assay (separately, determine the water ⟨*2.48*⟩ in the same manner as Ecabet Sodium Hydrate), dissolve in 1 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 271 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S.5H_2O$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 4500 \times 1.224$

M_S: Amount (mg) of ecabet sodium hydrate for assay taken, calculated on the anhydrous basis

M_T: Amount (g) of Ecabet Sodium Granules taken

C: Labeled amount (mg) of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S.5H_2O$) in 1 g

Assay Weigh accurately an amount of Ecabet Sodium Granules, equivalent to about 30 mg of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S.5H_2O$), add exactly 5 mL of the internal standard solution, add 25 mL of diluted methanol (1 in 2), shake vigorously for 20 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, to 3 mL of the subsequent filtrate add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ecabet sodium hydrate for assay (separately, determine the water ⟨*2.48*⟩ in the same manner as Ecabet Sodium Hydrate), add exactly 5 mL of the internal standard solution, and dissolve in dilute methanol (1 in 2) to make 30 mL. To 3 mL of this solution add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ecabet to that of the internal standard.

Amount (mg) of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S.5H_2O$)
$= M_S \times Q_T/Q_S \times 1.224$

M_S: Amount (mg) of ecabet sodium hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (1 in 2) (3 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.1 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 3.0. To 730 mL of this solution add 270 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ecabet is about 8 minutes.

System suitability—

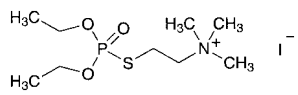
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ecabet and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ecabet to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Ecothiopate Iodide

エコチオパートヨウ化物



$C_9H_{23}INO_3PS$: 383.23
2-(Diethoxyphosphorylsulfanyl)-*N*,*N*,*N*-trimethylethylaminium iodide
[*513-10-0*]

Ecothiopate Iodide contains not less than 95.0% of ecothiopate iodide ($C_9H_{23}INO_3PS$), calculated on the dried basis.

Description Ecothiopate Iodide occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 0.1 g of Ecothiopate Iodide in 2 mL of water, and add 1 mL of nitric acid: a brown precipitate is formed. To 1 drop of the turbid solution containing this precipitate add 1 mL of hexane, and shake: a light red color develops in the hexane layer.

(2) Heat the suspension of the precipitate obtained in (1) until it becomes colorless, cool, add 10 mL of water, and use this solution as the sample solution. Two mL of the sample solution responds to the Qualitative Tests <*1.09*> (2) for phosphate.

(3) Two mL of the sample solution obtained in (2) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve 0.1 g of Ecothiopate Iodide in 40 mL of water: the pH of this solution is between 3.0 and 5.0.

Melting point <*2.60*> 116 – 122°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Ecothiopate Iodide in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—To 1.0 g of Ecothiopate Iodide in a Kjeldahl flask add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. Repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution becomes colorless, and white fumes are evolved. After cooling, transfer the solution together with a small quantity of water to a Nessler tube, and add water to make about 20 mL. Adjust the solution with ammonia solution (28) and ammonia TS to a pH between 3.0 and 3.5, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Ecothiopate Iodide in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 3 hours).

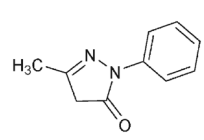
Assay Weigh accurately about 0.125 g of Ecothiopate Iodide, and dissolve in water to make exactly 100 mL. Pipet 10 mL of of this solution, add 30 mL of water, then add exactly 10 mL of phosphate buffer solution (pH 12), stopper the container, and allow to stand at 25 ± 3°C for 20 minutes. To this solution add quickly 2 mL of acetic acid (100), and titrate <*2.50*> with 0.002 mol/L iodine VS (potentiometric titration). Perform the test in the same manner without phosphate buffer solution (pH 12), and make any necessary correction.

Each mL of 0.002 mol/L iodine VS
= 1.533 mg of $C_9H_{23}INO_3PS$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and not exceeding 0°C.

Edaravone

エダラボン



$C_{10}H_{10}N_2O$: 174.20
5-Methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one
[*89-25-8*]

Edaravone, when dried, contains not less than 99.0% and not more than 101.0% of edaravone ($C_{10}H_{10}N_2O$).

Description Edaravone occurs as white to pale yellowish white, crystals or crystalline powder.

It is freely soluble in ethanol (99.5) and in acetic acid (100), and slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Edaravone (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Edaravone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> The pH of a solution obtained by dissolving 20 mg of Edaravone in 20 mL of water is between 4.0 and 5.5.

Melting point <*2.60*> 127 – 131°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Edaravone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Edaravone in

25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than edaravone obtained from the sample solution is not larger than the peak area of edaravone obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (100:100:1).

Flow rate: Adjust so that the retention time of edaravone is about 4 minutes.

Time span of measurement: About 7 times as long as the retention time of edaravone, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of edaravone are not less than 1500 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of edaravone is not more than 2.0%.

Loss on drying <2.41> Not more than 0.1% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Edaravone, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.42 mg of $C_{10}H_{10}N_2O$

Containers and storage Containers—Well-closed containers.

Edaravone Injection

エダラボン注射液

Edaravone Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of edaravone ($C_{10}H_{10}N_2O$: 174.20).

Method of preparation Prepare as directed under Injections, with Edaravone.

Description Edaravone Injection occurs as a clear and colorless liquid.

Identification To a volume of Edaravone Injection, equivalent to 1.5 mg of Edaravone, add water to make 50 mL. To 5 mL of this solution add water to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 238 nm and 242 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Related substance—(i) Use Edaravone Injection as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than edaravone obtained from the sample solution is not larger than 2 times the peak area of edaravone obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Edaravone.

Time span of measurement: About 7 times as long as the retention time of edaravone, beginning after the peak of edaravone.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of edaravone are not less than 1500 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of edaravone is not more than 2.0%.

(ii) Use Edaravone Injection as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 to edaravone, obtained from the sample solution is not larger than 4 times the peak area of edaravone obtained from the standard solution, the area of the peak, having the relative retention time of about 0.4 to edaravone, is not larger than the peak area of edaravone from the standard solution, and the area of the peak other than edaravone and the peaks mentioned above is not larger than 2 times the peak area of edaravone from the standard solution.

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Column temperature: A constant temperature of about 40°C.

Flow rate: Adjust so that the retention time of edaravone is about 11 minutes.

Time span of measurement: About 2.5 times as long as the retention time of edaravone, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of edaravone are not less than 2000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of edaravone is not more than 2.0%.

Bacterial endotoxins ⟨*4.01*⟩ Less than 5.0 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Edaravone Injection, equivalent to about 3 mg of edaravone ($C_{10}H_{10}N_2O$) add exactly 10 mL of the internal standard solution, add methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of edaravone for assay, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of edaravone to that of the internal standard.

$$\text{Amount (mg) of edaravone } (C_{10}H_{10}N_2O) = M_S \times Q_T/Q_S \times 1/25$$

M_S: Amount (mg) of edaravone for assay taken

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted dilute acetic acid (1 in 100) and methanol (3:1), adjusted to pH 5.5 with diluted ammonia solution (28) (1 in 20).

Flow rate: Adjust so that the retention time of edaravone is about 8 minutes.

System suitability—

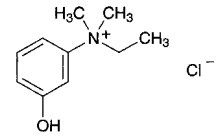
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, edaravone and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of edaravone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Edrophonium Chloride

エドロホニウム塩化物



$C_{10}H_{16}ClNO$: 201.69

N-Ethyl-3-hydroxy-*N*,*N*-dimethylanilinium chloride

[*116-38-1*]

Edrophonium Chloride, when dried, contains not less than 98.0% of edrophonium chloride ($C_{10}H_{16}ClNO$).

Description Edrophonium Chloride occurs as white, crystals or crystalline powder. It is odorless.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

It is hygroscopic.

It is gradually colored by light.

Identification **(1)** To 5 mL of a solution of Edrophonium Chloride (1 in 100) add 1 drop of iron (III) chloride TS: a light red-purple color develops.

(2) Determine the absorption spectrum of a solution of Edrophonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Edrophonium Chloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Edrophonium Chloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point ⟨*2.60*⟩ 166 – 171°C (with decomposition).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Edrophonium Chloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonia solution (28) (16:4:1) to a distance of about 10 cm, and air-dry

the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.20% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Edrophonium Chloride, previously dried, and dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.17 mg of $C_{10}H_{16}ClNO$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Edrophonium Chloride Injection

エドロホニウム塩化物注射液

Edrophonium Chloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of edrophonium chloride ($C_{10}H_{16}ClNO$: 201.69).

Method of preparation Prepare as directed under Injections, with Edrophonium Chloride.

Description Edrophonium Chloride Injection is a clear and colorless liquid.

Identification **(1)** To a volume of Edrophonium Chloride Injection, equivalent to 0.04 g of Edrophonium Chloride, add 4 mL of barium nitrate TS, shake, and filter. Proceed with the filtrate as directed in the Identification (1) under Edrophonium Chloride.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 272 nm and 276 nm.

pH ⟨*2.54*⟩ 6.5 – 8.0

Bacterial endotoxins ⟨*4.01*⟩ Less than 15 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Measure exactly a volume of Edrophonium Chloride Injection, equivalent to about 50 mg of edrophonium chloride ($C_{10}H_{16}ClNO$), place in a chromatographic column prepared by pouring 10 mL of weakly basic DEAE-bridged dextran anion exchanger (Cl type) (50 to 150 μm in particle diameter) into a chromatographic tube about 2 cm in inside diameter and about 10 cm in length, add 25 mL of water, and elute at the flow rate of 1 to 2 mL per minute. Wash the column with two 25-mL portions of water at the flow rate of 1 to 2 mL per minute. Combine the washings with above effluent solutions, and add water to make exactly 100 mL. Measure exactly 10 mL of this solution, and add 10 mL of phosphate buffer solution (pH 8.0) and 5 g of sodium chloride. Wash this solution with four 20-mL portions of a mixture of diethyl ether and hexane (1:1), collect the water layer, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Edrophonium Chloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 3 hours, and dissolve in water to make exactly 100 mL. Measure exactly 10 mL of this solution, and prepare the standard solution in the same manner as the sample solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

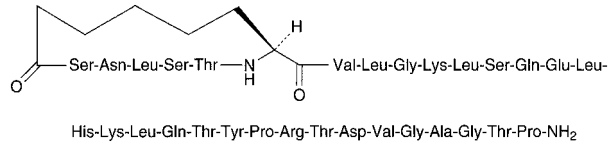
Amount (mg) of edrophonium chloride ($C_{10}H_{16}ClNO$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Edrophonium Chloride RS taken

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Elcatonin

エルカトニン



His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asp-Val-Gly-Ala-Gly-Thr-Pro-NH_2

$C_{148}H_{244}N_{42}O_{47}$: 3363.77
[*60731-46-6*]

Elcatonin contains not less than 5000 Elcatonin Units and not more than 7000 Elcatonin Units per mg of peptide, calculated on the anhydrous and residual acetic acid-free basis.

Description Elcatonin is a white powder.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

The pH of its solution (1 in 500) is between 4.5 and 7.0.

Identification Dissolve 5 mg of Elcatonin in 5 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Constituent amino acids Put about 1 mg of Elcatonin in a test tube for hydrolysis, add phenol-hydrochloric acid TS to dissolve, replace the air inside with Nitrogen, seal the tube under reduced pressure, and heat at 110 ± 2°C for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in about 1 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh exactly 1.33 mg of L-aspartic acid, 1.19 mg of L-threonine, 1.05 mg of L-serine, 1.47 mg of L-glutamic acid, 1.15 mg of L-proline,

0.75 mg of glycine, 0.89 mg of L-alanine, 1.17 mg of L-valine, 1.89 mg of L-2-aminosuberic acid, 1.31 mg of L-leucine, 1.81 mg of L-tyrosine, 1.83 mg of L-lysine hydrochloride, 2.10 mg of L-histidine hydrochloride monohydrate and 2.11 mg of L-arginine hydrochloride, dissolve them in 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exaxtly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: 14 peaks of amino acids appear on the chromatogram obtained from the sample solution, and their respective molar ratios against alanine are 1.7 – 2.2 for aspartic acid, 3.5 – 4.2 for threonine, 2.4 – 3.0 for serine, 2.7 – 3.2 for glutamic acid, 1.7 – 2.2 for proline, 2.7 – 3.2 for glycine, 1.6 – 2.2 for valine, 0.8 – 1.2 for 2-aminosuberic acid, 4.5 – 5.2 for leucine, 0.7 – 1.2 for tyrosine, 1.7 – 2.2 for lysine, 0.8 – 1.2 for histidine and 0.7 – 1.2 for arginine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated styrene-divinylbenzene copolymer (3 μm in particle diameter).

Column temperature: Varied between 50°C and 65°C.

Chemical reaction vessel temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Buffer solutions A, B, C and D, with sodium ion concentrations of 0.10 mol/L, 0.135 mol/L, 1.26 mol/L and 0.20 mol/L, respectively. The ion concentration of the mobile phase is changed stepwise from 0.10 mol/L to 1.26 mol/L by using these buffer solutions.

Components of buffer solutions

Buffer solution:	A	B	C	D
Citric acid monohydrate	8.85 g	7.72 g	6.10 g	—
Trisodium citrate dihydrate	3.87 g	10.05 g	26.67 g	—
Sodium hydroxide	—	—	2.50 g	8.00 g
Sodium chloride	3.54 g	1.87 g	54.35 g	—
Ethanol (95)	60.0 mL	—	—	60.0 mL
Thiodiglycol	5.0 mL	5.0 mL	—	—
Purified water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	1000 mL	1000 mL	1000 mL	1000 mL

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for about 20 minutes while passing Nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for about 20 minutes while passing Nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: Adjust so that the retention time of arginine is about 75 minutes.

Flow rate of reaction reagent: About 0.2 mL per minute.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column from which aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, 2-aminosuberic acid, leucine, tyrosine, lysine, histidine and arginine are eluted in this order, with complete separation of each peak.

Purity **(1)** Acetic acid—Weigh accurately 3 – 6 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, add exactly 1 mL of the internal standard solution to dissolve it, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetic acid (100), and add the internal standard solution to make exactly 100 mL. Pipet 5 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not more than 7.0%.

$$\text{Amount (\%) of acetic acid } (CH_3COOH) = M_{ST}/M_{SA} \times Q_T/Q_S \times 50$$

M_{ST}: Amount (g) of acetic acid (100) taken

M_{SA}: Amount (mg) of Elcatonin taken

Internal standard solution—A solution of citric acid monohydrate (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 13.2 g of diammonium hydrogen phosphate in 900 mL of water, add phosphoric acid to adjust the pH to 2.5, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of acetic acid is about 4 minutes.

Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions. Use a column from which acetic acid and citric acid are eluted in this order with the resolution between their peaks being not less than 2.0.

(2) Related substances—Dissolve 1.0 mg of Elcatonin in 1 mL of a mixture of trifluoroacetic acid TS and acetonitrile (2:1), and use this solution as the sample solution. Take exactly 0.3 mL of the sample solution, add a mixture of trifluoroacetic acid TS and acetonitrile (2:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than elcatonin from the sample solution is not larger than 1/3 times the peak area of elcatonin from the standard solution, and the total of the peak areas other than elcatonin is not larger than the peak area of elcatonin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

40°C.

Mobile phase: A mixture of trifluoroacetic acid TS and acetonitrile (change the ratio linearly from 85:15 to 55:45 in 30 minutes).

Flow rate: Adjust so that the retention time of elcatonin is about 25 minutes.

Selection of column: Dissolve 2 mg of Elcatonin in 200 μL of trypsin TS for test of elcatonin, warm at 37°C for 1 hour, then add 1 drop of acetic acid (100), and heat at 95°C for 1 minute. To 10 μL of this solution add 50 μL of the sample solution, and mix. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column such that the resolution between the peak of elcatonin and the peak which appears immediately before the peak of elcatonin is not less than 2.0, and the retention time of elcatonin is about 25 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of elcatonin from 10 μL of the standard solution is between 50 mm and 200 mm.

Time span of measurement: Continue measurement until the regularly changing base-line of the chromatogram disappears, beginning after the solvent peak.

Water ⟨*2.48*⟩ Weigh accurately 1 – 3 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and perform the test as directed in Coulometric titration: not more than 8.0%.

Nitrogen content Weigh accurately 0.015 – 0.02 g of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩: it contains not less than 16.1% and not more than 18.7% of nitrogen (N: 14.01) in the peptide, calculated on the anhydrous and residual acetic acid-free basis.

Assay (i) Animals: Select healthy male Sprague-Dawley rats each weighing between 90 g and 110 g. Keep the rats for not less than 3 days before use, providing an appropriate uniform diet and water.

(ii) Diluent for elcatonin: Dissolve 2.72 g of sodium acetate trihydrate in water to make 200 mL, add 0.2 g of bovine serum albumin, and adjust the pH to 6.0 with acetic acid (100). Prepare before use.

(iii) Standard solution: Dissolve Elcatonin RS in the diluent for elcatonin to make two standard solutions, one to contain exactly 0.075 Unit in each mL which is designated as the high-dose standard solution, S_H, and the other to contain exactly 0.0375 Unit in each mL which is designated as the low-dose standard solution, S_L.

(iv) Sample solution: Weigh accurately 0.5 – 2.0 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and dissolve in the diluent for elcatonin to make two sample solutions, the high-dose sample solution, T_H, which contains the Units per mL equivalent to S_H and the low-dose sample solution, T_L, which contains the Units per mL equivalent to S_L.

(v) Deproteinizing solution for elcatonin: Dissolve 160 g of trichloroacetic acid and 30.6 g of strontium chloride in water to make 3600 mL.

(vi) Procedure: Divide the animals into 4 equal groups of not less than 10 animals each. Withhold all food, but not water, for 18 to 24 hours before the injections, and withhold water during the assay until the final blood sample is taken. Handle the animals with care in order to avoid undue excitement.

Inject exactly 0.2 mL each of the standard solutions and the sample solutions into the tail vein of each animal as indicated in the following design:

First group	S_H	Third group	T_H
Second group	S_L	Fourth group	T_L

At 1 hour after the injection, take a sufficient blood sample to perform the test from the carotid artery and vein of each animal under ether anesthesia, centrifuge the blood samples to separate serum, and determine the serum calcium according to the following (vii).

(vii) Serum calcium determination: Take exactly 0.3 mL of the serum, add the deproteinizing solution for elcatonin to make exactly 3 mL, mix well, centrifuge, and use the supernatant liquid as the sample solution for calcium determination. Separately, pipet 1 mL of Standard Calcium Solution for Atomic Absorption Spectrophotometry, and add a solution of sodium chloride (17 in 2000) to make exactly 10 mL. Pipet 5 mL of this solution, add the deproteinizing solution for elcatonin to make exactly 50 mL, and use this solution as the standard solution for calcium determination. Determine the absorbances, A_T and A_S, of the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions. Determine the absorbance, A_0, of a solution obtained in the same manner used for preparation of the standard solution, but with 1 mL of water instead of the standard solution.

Amount (mg) of calcium (Ca) in 100 mL of the serum
$= 0.01 \times (A_T - A_0)/(A_S - A_0) \times 10 \times 100$

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Calcium hollow-cathode lamp.
Wavelength: 422.7 nm.

(viii) Calculation: Amounts of calcium in 100 mL of the serum obtained with S_H, S_L, T_H and T_L in (vii) are symbolized as y_1, y_2, y_3 and y_4, respectively. Sum up individual y_1, y_2, y_3 and y_4 to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

Units per mg of peptide, calculated on the anhydrous and residual acetic acid-free basis
$=$ antilog $M \times$ units per mL of $S_H \times b/a$

$M = 0.3010 \times Y_a/Y_b$

$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$
$Y_b = Y_1 - Y_2 + Y_3 - Y_4$

a: Amount (mg) of Elcatonin taken

$\times$ [100 − {water content (%) + acetic acid content (%)}/100]

b: Total volume (mL) of the high-dose sample solution prepared by dissolving Elcatonin with diluent for elcatonin

F' computed by the following equation should be smaller than F shown in the table against n with which s^2 is calculated. Calculate L (P = 0.95) by use of the following equation: L should be not more than 0.20. If F' exceeds F, or if L exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that F' is not more than F and L is not more than 0.20.

$$F' = (-Y_1 + Y_2 + Y_3 - Y_4)^2/4fs^2$$

f: Number of the animals of each group

$$s^2 = \{\Sigma y^2 - (Y/f)\}/n$$

Σy^2: The sum of squares of y_1, y_2, y_3 and y_4 in each group
$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$
$n = 4(f - 1)$

$$L = 2\sqrt{(C-1)(CM^2 + 0.09062)}$$

$$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$$

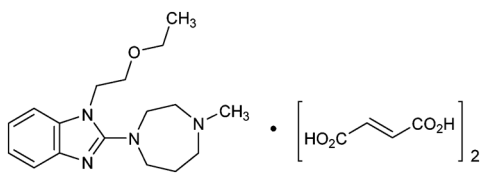
t^2: Value shown in the following table against n used to calculate s^2

n	$t^2 = F$	n	$t^2 = F$	n	$t^2 = F$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.
Storage—Not exceeding 8°C.

Emedastine Fumarate

エメダスチンフマル酸塩



$C_{17}H_{26}N_4O.2C_4H_4O_4$: 534.56
1-(2-Ethoxyethyl)-2-(4-methyl-1,4-diazepan-1-yl)-1*H*-benzimidazole difumarate
[*87233-62-3*]

Emedastine Fumarate, when dried, contains not less than 98.5% and not more than 101.0% of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$).

Description Emedastine Fumarate occurs as a white to pale yellow crystalline powder.

It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in acetic acid (100).

It shows crystal polymorphism.

Identification **(1)** Dissolve 10 mg of Emedastine Fumarate in 10 mL of water. To 2 mL of this solution add 1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Emedastine Fumarate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 30 mg of Emedastine Fumarate in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of fumaric acid for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, formic acid and water (90:7:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the spot on the starting point from the sample solution and the spot from the standard solution show the same Rf value.

Melting point <*2.60*> 149 – 152°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Emedastine Fumarate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 10 mg of Emedastine Fumarate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than emedastine and fumaric acid obtained from the sample solution is not larger than the peak area of emedastine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.9 g of sodium dihydrogen phosphate dihydrate and 2.5 g of sodium lauryl sulfate in 1000 mL of water, and adjust to pH 2.4 with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust so that the retention time of emedastine is about 18 minutes.

Time span of measurement: About 2 times as long as the retention time of emedastine, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of emedastine are not less than 10,000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of emedastine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Emedastine Fumarate, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.73 mg of $C_{17}H_{26}N_4O.2C_4H_4O_4$

Containers and storage Containers—Tight containers.

Emedastine Fumarate Extended-release Capsules

エメダスチンフマル酸塩徐放カプセル

Emedastine Fumarate Extended-release Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$: 534.56).

Method of preparation Prepare as directed under Capsules, with Emedastine Fumarate.

Identification (1) Powder the content of Emedastine Fumarate Extended-release Capsules. To a portion of the powder, equivalent to 10 mg of Emedastine Fumarate, add 10 mL of water, shake thoroughly, and filter. Spot 1 drop of the filtrate on a filter paper, and spray Dragendorff's TS for spraying on the filter: the spot shows an orange color.

(2) To 2 mL of the filtrate obtained in (1) add 1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 278 nm and 282 nm, and between 284 nm and 288 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Emedastine Fumarate Extended-release Capsules add 40 mL of the mobile phase, agitate with the aid of ultrasonic waves for 30 minutes while occasional vigorous shaking, and add the mobile phase to make exactly V mL so that each mL contains about 20 μg of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$). Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of emedastine fumarate for assay taken

Internal standard solution—A solution of 4-methyl-benzophenone in the mobile phase (1 in 40,000).

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately the content of not less than 20 Emedastine Fumarate Extended-release Capsules, and powder. Weigh accurately a portion of the powder, equivalent to about 2 mg of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$), add 10 mL of the mobile phase, agitate with the aid of ultrasonic waves for 30 minutes while occasional vigorous shaking, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of emedastine fumarate for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make 100 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 50 mL. Then, pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>, according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of emedastine to that of the internal standard.

Amount (mg) of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of emedastine fumarate for assay taken

Internal standard solution—A solution of 4-methylbenzophenone in the mobile phase (1 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.9 g of sodium dihydrogen phosphate dihydrate and 2.5 g of sodium lauryl sulfate in 1000 mL of water, and adjust to pH 2.4 with phosphoric acid. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of emedastine is about 6 minutes.

System suitability—

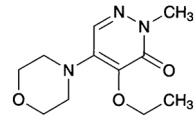
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, emedastine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of emedastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Emorfazone

エモルファゾン



$C_{11}H_{17}N_3O_3$: 239.27
4-Ethoxy-2-methyl-5-(morpholin-4-yl)pyridazin-3(2*H*)-one
[*38957-41-4*]

Emorfazone, when dried, contains not less than 98.5% and not more than 101.0% of emorfazone ($C_{11}H_{17}N_3O_3$).

Description Emorfazone occurs as colorless crystals or a white to light yellow crystalline powder.

It is very soluble in ethanol (99.5), and freely soluble in water and in acetic anhydride.

It dissolves in 1 mol/L hydrochloric acid TS.

It gradually turns yellow and decomposes on exposure to light.

Identification **(1)** Dissolve 20 mg of Emorfazone in 2 mL of 1 mol/L hydrochloric acid TS, and add 5 drops of Reinecke's TS: light red floating matters are formed.

(2) Determine the absorption spectrum of a solution of Emorfazone (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Emorfazone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 89 – 92°C (after drying).

Purity **(1)** Chloride <1.03>—Perform the test with 1.0 g of Emorfazone. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Emorfazone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Emorfazone according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.5 g of Emorfazone in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than emorfazone obtained from the sample solution is not larger than 1/10 times the peak area of emorfazone obtained from the standard solution, and the total area of the peaks other than emorfazone from the sample solution is not larger than 1/2 times the peak area of emorfazone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (11:10).

Flow rate: Adjust so that the retention time of emorfazone is about 5 minutes.

Time span of measurement: About 2.5 times as long as the retention time of emorfazone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of emorfazone obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the standard solution.

System performance: Dissolve 16 mg of Emorfazone and 30 mg of 2,4-dinitrophenylhydrazine in 100 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, emorfazone and 2,4-dinitrophenylhydrazine are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of emorfazone is not more than 1.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Emorfazone, previously dried, dissolve in 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.93 mg of $C_{11}H_{17}N_3O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Emorfazone Tablets

エモルファゾン錠

Emorfazone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of emorfazone ($C_{11}H_{17}N_3O_3$: 239.27).

Method of preparation Prepare as directed under Tablets, with Emorfazone.

Identification To a quantity of powdered Emorfazone Tablets, equivalent to 0.1 g of Emorfazone, add 100 mL of water, shake well, and centrifuge. Filter the supernatant liquid, and to 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 237 nm and 241 nm, and between 310 nm and 314 nm, and a shoulder between 288 nm and 298 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Emorfazone Tablets add methanol to make exactly V mL so that each mL contains about 4 mg of emorfazone ($C_{11}H_{17}N_3O_3$), and shake well to disintegrate. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of emorfazone ($C_{11}H_{17}N_3O_3$)
$= M_S \times Q_T/Q_S \times V/5$

M_S: Amount (mg) of emorfazone for assay taken

Internal standard solution—A solution of 2,4-dinitrophenylhidrazine in methanol (3 in 2000). Prepare before use.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Emorfazone Tablets is not less than 80%.

Start the test with 1 tablet of Emorfazone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the

first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of emorfazone ($C_{11}H_{17}N_3O_3$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of emorfazone for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of emorfazone ($C_{11}H_{17}N_3O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of emorfazone for assay taken
C: Labeled amount (mg) of emorfazone ($C_{11}H_{17}N_3O_3$) in 1 tablet

Assay To 10 tablets of Emorfazone Tablets add 200 mL of methanol, shake well to disintegrate, add methanol to make exactly 250 mL, and centrifuge. Pipet a volume of the supernatant liquid, equivalent to about 8 mg of emorfazone ($C_{11}H_{17}N_3O_3$), add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of emorfazone for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of emorfazone to that of the internal standard.

Amount (mg) of emorfazone ($C_{11}H_{17}N_3O_3$)
$= M_S \times Q_T/Q_S \times 2/5$

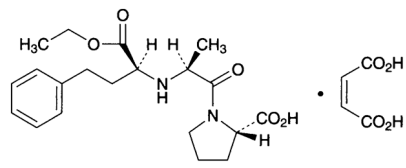
M_S: Amount (mg) of emorfazone for assay taken

Internal standard solution—A solution of 2,4-dinitrophenylhidrazine in methanol (3 in 2000). Prepare before use.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 313 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water and methanol (11:10).
Flow rate: Adjust so that the retention time of emorfazone is about 5 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, emorfazone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of emorfazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Enalapril Maleate

エナラプリルマレイン酸塩



$C_{20}H_{28}N_2O_5.C_4H_4O_4$: 492.52
(2*S*)-1-{(2*S*)-2-[(1*S*)-1-Ethoxycarbonyl-3-phenylpropylamino]propanoyl}pyrrolidine-2-carboxylic acid monomaleate
[*76095-16-4*]

Enalapril Maleate, when dried, contains not less than 98.0% and not more than 102.0% of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$).

Description Enalapril Maleate occurs as white crystals or a white crystalline powder.
It is freely soluble in methanol, sparingly soluble in water and in ethanol (99.5), and slightly soluble in acetonitrile.
Melting point: about 145°C (with decomposition).

Identification **(1)** Determine the infrared absorption spectra of Enalapril Maleate as directed in the potassium bromide disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Enalapril Maleate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
(2) To 20 mg of Enalapril Maleate add 5 mL of 1 mol/L hydrochloric acid TS, shake, add 5 mL of diethyl ether, and shake for 5 minutes. Take 3 mL of the upper layer, distil off the diethyl ether on a water bath, add 5 mL of water to the residue with shaking, and add 1 drop of potassium permanganate TS: the red color of the test solution immediately disappears.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -41.0 – $-43.5°$ (after drying, 0.25 g, methanol, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Enalapril Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(2) Related substances—Dissolve 30 mg of Enalapril Maleate in 100 mL of a mixture of sodium dihydrogen phosphate TS (pH 2.5) and acetonitrile (19:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of sodium dihydrogen phosphate TS (pH 2.5) and acetonitrile (19:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> acccording to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than maleic acid and enalapril obtained from the sample solution is not larger than the peak area of enalapril obtained from the standard solution. Furthermore, the total area of the peaks other than maleic acid and enalapril from the sample solution is not larger than 2 times the peak area of enalapril from the standard solution.
Operating conditions—
Detector, column, column temperature, mobile phases, flowing of mobile phase, and flow rate: Proceed as directed

in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of enalapril, beginning after the peak of maleic acid.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of sodium dihydrogen phosphate TS (pH 2.5) and acetonitrile (19:1) to make exactly 10 mL. Confirm that the peak area of enalapril obtained from 50 μL of this solution is equivalent to 7 to 13% of that obtained from 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Enalapril Maleate and Enalapril Maleate RS, both previously dried, and dissolve in a mixture of sodium dihydrogen phosphate TS (pH 2.5) and acetonitrile (19:1) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of enalapril in each solution.

Amount (mg) of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Enalapril Maleate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 15 cm in length, packed with porous styrene-divinylbenzene copolymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 70°C.

Mobile phase A: Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 6.8 with a solution of sodium hydroxide (1 in 4), and add water to make 1000 mL. To 950 mL of this solution, add 50 mL of acetonitrile for liquid chromatography.

Mobile phase B: Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 6.8 with a solution of sodium hydroxide (1 in 4), and add water to make 1000 mL. To 340 mL of this solution, add 660 mL of acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the concentration gradient by changing the ratio of the mobile phases A and B as follows.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0	95	5
0 – 20	95 → 40	5 → 60
20 – 25	40	60

Flow rate: 1.4 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of enalapril is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Enalapril Maleate Tablets

エナラプリルマレイン酸塩錠

Enalapril Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$: 492.52).

Method of preparation Prepare as directed under Tablets, with Enalapril Maleate.

Identification To a quantity of powdered Enalapril Maleate Tablets, equivalent to 50 mg of Enalapril Maleate, add 20 mL of methanol, shake, centrifuge, and then use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of enalapril maleate in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-Layer Chromatography ⟨*2.03*⟩. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of water, acetone, 1-butanol, acetic acid (100) and toluene (1:1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f values of the 2 spots obtained from the sample solution and the 2 spots obtained from the standard solution are equivalent.

Purity Enalaprilat and enalapril diketopiperazine—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add sodium dihydrogen phosphate TS (pH 2.2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the peak area of enalaprilat, having the relative retention time of about 0.5 to enalapril obtained from the sample solution, is not larger than 2 times the peak area of enalapril obtained from the standard solution. Also, the peak area of enalapril diketopiperazine, having the relative retention time of about 1.5 to enalapril, from the sample so-

lution is not larger than the peak area of enalapril from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the standard solution, and add sodium dihydrogen phosphate TS (pH 2.2) to make exactly 10 mL. Confirm that the peak area of enalapril obtained from 50 μL of this solution is equivalent to 7 to 13% of that obtained from 50 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Enalapril Maleate Tablets, add V/2 mL of sodium dihydrogen phosphate TS (pH 2.2), treat with ultrasonic waves for 15 minutes, shake for 30 minutes, and add sodium dihydrogen phosphate TS (pH 2.2) to make exactly V mL so that each mL contains about 0.1 mg of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$). Treat this solution with ultrasonic waves for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of Enalapril Maleate RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of a 2.5- and 5-mg tablet and in 30 minutes of a 10-mg tablet are not less than 85%, respectively.

Start the test with 1 tablet of Enalapril Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 14 mg of Enalapril Maleate RS, previously dried in vacuum at 60°C for 2 hours, and dissolve in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of enalapril in each solution.

Dissolution rate (%) with respect to the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Enalapril Maleate RS taken

C: Labeled amount (mg) of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$) in 1 tablet

Operating conditions—

Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 1.88 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 2.2 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 250 mL of acetonitrile.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril are not less than 300 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.

Assay Weigh accurately not less than 20 Enalapril Maleate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$), add 50 mL of sodium dihydrogen phosphate TS (pH 2.2), treat with ultrasonic waves for 15 minutes, shake for 30 minutes, and then add sodium dihydrogen phosphate TS (pH 2.2) to make exactly 100 mL. Treat this solution with ultrasonic waves for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Enalapril Maleate RS, previously dried in vacuum at 60°C for 2 hours, dissolve in sodium dihydrogen phosphate TS (pH 2.2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of enalapril in each solution.

Amount (mg) of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Enalapril Maleate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of sodium dihydrogen phosphate TS (pH 2.2) and acetonitrile (3:1).

Flow rate: Adjust so that the retention time of enalapril is about 5 minutes.

System suitability—

System performance: Heat to fusion about 20 mg of enalapril maleate. After cooling, add 50 mL of acetonitrile, and treat with ultrasonic waves to dissolve. To 1 mL of this solution, add the standard solution to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 50 μL of the solution for system suitability test under the above operaring conditions, enalapril and enalapril diketopiperazine, which has a relative retention time of about 1.5 to enalapril, are eluted in this order with the resolution between these peaks being not less than 2.0.

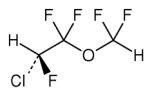
System repeatability: When the test is repeated 6 times with 50 μL of the solution for system suitability test under

the above operating conditions, the relative standard deviation of the peak area of enalapril is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Enflurane

エンフルラン



and enantiomer

$C_3H_2ClF_5O$: 184.49
(2*RS*)-2-Chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane
[*13838-16-9*]

Description Enflurane is a clear, colorless liquid.
It is slightly soluble in water.
It is miscible with ethanol (95) and with diethyl ether.
It is a volatile, and not an inflammable.
It shows no optical rotation.
Boiling point: 54 – 57°C

Identification **(1)** Take 50 μL of Enflurane, and prepare the test solution as directed to the Oxygen Flask Combustion Method ⟨*1.06*⟩ using 40 mL of water as the absorbing liquid. The test solution responds to the Qualitative Tests ⟨*1.09*⟩ for chloride and fluoride.

(2) Determine the infrared absorption spectrum of Enflurane as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.302 – 1.304

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.520 – 1.540

Purity **(1)** Acidity or alkalinity—To 60 mL of Enflurane add 60 mL of freshly boiled and cooled water, shake for 3 minutes, separate the water later, and use the layer as the sample solution. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is purple. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.06 mL of 0.01 mol/L hydrochloric acid VS: the color of the solution is yellow.

(2) Chloride ⟨*1.03*⟩—To 20 g of Enflurane add 20 mL of water, shake well, and separate the water layer. Take 10 mL of the water layer add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001%).

(3) Related substances—Proceed the test with 5 μL of Enflurane as directed under Gas chromatography ⟨*2.02*⟩ according to the following conditions. Determine each peak area other than the peak of air which appears soon after injection of the sample by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of the substances other than enflurane is not more than 0.10%.

Operating conditions—

Detector: A thermal conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with diethylene glycol succinate ester for gas chromatography in the ratio of 20%.

Column temperature: A constant temperature of about 80°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of enflurane is about 3 minutes.

Time span of measurement: About 3 times as long as the retention time of enflurane.

System suitability—

Test for required detectability: To exactly 1 mL of enflurane add 2-propanol to make exactly 100 mL. To exactly 2 mL of this solution add 2-propanol to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add 2-propanol to make exactly 10 mL. Confirm that the peak area of enflurane obtained from 5 μL of this solution is equivalent to 7 to 13% of that obtained from 5 μL of the solution for system suitability test.

System performance: Mix 5 mL of Enflurane and 5 mL of 2-propanol. When the procedure is run with 5 μL of this mixture under the above operating conditions, enflurane and 2-propanol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of enflurane is not more than 2.0%.

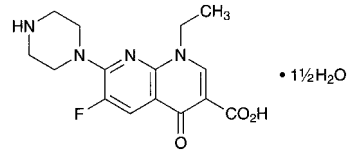
(4) Nonvolatile residue—Evaporate exactly 65 mL of Enflurane on a water bath to dryness, and dry the residue at 105°C for 1 hour: the residue is not more than 1.0 mg.

Water ⟨*2.48*⟩ Not more than 0.10% (10 g, volumetric titration, direct titration).

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Enoxacin Hydrate

エノキサシン水和物



$C_{15}H_{17}FN_4O_3.1\frac{1}{2}H_2O$: 347.34
1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid sesquihydrate
[*84294-96-2*]

Enoxacin Hydrate, when dried, contains not less than 98.5% of enoxacin ($C_{15}H_{17}FN_4O_3$: 320.32).

Description Enoxacin Hydrate occurs as white to pale yellow-brown, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol, very slightly soluble in chloroform, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS.

It is gradually colored by light.

Identification **(1)** Place 0.02 g of Enoxacin Hydrate and 0.05 g of sodium in a test tube, and heat gradually to ignition with precaution. After cooling, add 0.5 mL of methanol and then 5 mL of water, and heat to boiling. To this solution add 2 mL of dilute acetic acid, and filter: the filtrate responds to

the Qualitative Tests ⟨*1.09*⟩ (2) for fluoride.

(2) Dissolve 0.05 g of Enoxacin Hydrate in dilute sodium hydroxide TS to make 100 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Enoxacin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 225 – 229°C (after drying).

Purity **(1)** Sulfate ⟨*1.14*⟩—Dissolve 1.0 g of Enoxacin Hydrate in 50 mL of dilute sodium hydroxide TS, shake with 10 mL of dilute hydrochloric acid, and centrifuge. Filter the supernatant liquid, and to 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 25 mL of dilute sodium hydroxide TS, 5 mL of dilute hydrochloric acid TS and water to make 50 mL (not more than 0.048%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Enoxacin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Enoxacin Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Enoxacin Hydrate in 25 mL of a mixture of chloroform and methanol (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (7:3) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ 7.0 – 9.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g, platinum crucible).

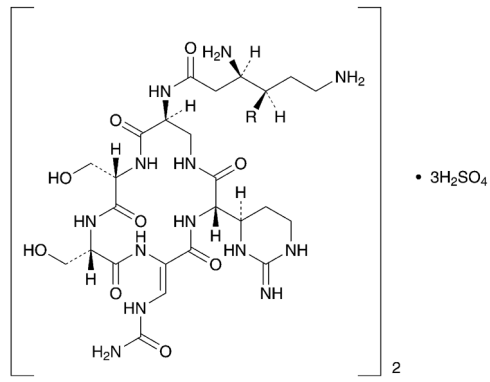
Assay Weigh accurately about 0.3 g of Enoxacin Hydrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.03 mg of $C_{15}H_{17}FN_4O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Enviomycin Sulfate

エンビオマイシン硫酸塩



Tuberactinomycin N : R = OH
Tuberactinomycin O : R = H

Tuberactinomycin N Sulfate
$(C_{25}H_{43}N_{13}O_{10})_2.3H_2SO_4$: 1665.62
Tuberactinomycin O Sulfate
$(C_{25}H_{43}N_{13}O_9)_2.3H_2SO_4$: 1633.62
Tuberactinomycin N Sulfate
(3*R*,4*R*)-*N*-[(3*S*,9*S*,12*S*,15*S*)-9,12-Bis(hydroxymethyl)-3-[(4*R*)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaoxo-6-(*Z*)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diamino-4-hydroxyhexanamide sesquisulfate
[*33103-22-9*, Tuberactinomycin N]

Tuberactinomycin O Sulfate
(3*S*)-*N*-[(3*S*,9*S*,12*S*,15*S*)-9,12-Bis(hydroxymethyl)-3-[(4*R*)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaoxo-6-(*Z*)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diaminohexanamide sesquisulfate
[*33137-73-4*, Tuberactinomycin O]

Enviomycin Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Streptomyces griseoverticillatus* var. *tuberacticus*.

It contains not less than 770 μg (potency) and not more than 920 μg (potency) per mg, calculated on the dried basis. The potency of Enviomycin Sulfate is expressed as mass (potency) of tuberactinomycin N ($C_{25}H_{43}N_{13}O_{10}$: 685.69).

Description Enviomycin Sulfate occurs as a white powder.
It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** To 5 mL of a solution of Enviomycin Sulfate (1 in 200) add 1.5 mL of sodium hydroxide TS, and add 1 drop of a mixture of 0.01 mol/L citric acid TS and copper (II) sulfate TS (97:3) : a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Enviomycin Sulfate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 2 mL of a solution of Enviomycin Sulfate (1 in 20) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −16 – −22° (0.5 g calculated

on the dried basis, water, 50 mL, 100 mm).

pH ⟨2.54⟩ The pH of a solution obtained by dissolving 2.0 g of Enviomycin Sulfate in 20 mL of water is between 5.5 and 7.5.

Content ratio of the active principle Dissolve 0.1 g of Enviomycin Sulfate in water to make 100 mL, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the peak areas, A_{T1} and A_{T2}, of tuberactinomycin N and tuberactinomycin O, having the relative retention time, 1.4 ± 0.4, to tuberactinomycin N, by the automatic integration method: $A_{T2}/(A_{T1} + A_{T2})$ is between 0.090 and 0.150.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ammonium acetate TS, 1,4-dioxane, tetrahydrofuran, water and ammonia solution (28) (100:75:50:23:2).

Flow rate: Adjust so that the retention time of tuberactinomycin N is about 9 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the sample solution under the above operating conditions, tuberactinomycin N and tuberactinomycin O are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 3 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of tuberactinomycin N is not more than 2.0%.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Enviomycin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨1.07⟩—Proceed with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨1.11⟩—Prepare the test solution with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying ⟨2.41⟩ Not more than 4.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨4.02⟩ according to the following conditions.

(i) Test organism—*Bacillius subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

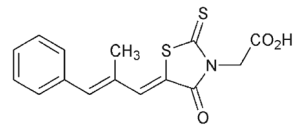
(iii) Standard solutions—Weigh accurately an amount of Enviomycin Sulfate RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 400 μg (potency) and 100 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Enviomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 400 μg (potency) and 100 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Epalrestat

エパルレスタット



$C_{15}H_{13}NO_3S_2$: 319.40

2-{(5*Z*)-5-[(2*E*)-2-Methyl-3-phenylprop-2-en-1-ylidene]-4-oxo-2-thioxothiazolidin-3-yl}acetic acid

[*82159-09-9*]

Epalrestat, when dried, contains not less than 98.0% and not more than 101.0% of epalrestat ($C_{15}H_{13}NO_3S_2$).

Description Epalrestat occurs as yellow to orange, crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It gradually fades the color and decomposes on exposure to light.

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Epalrestat in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Epalrestat RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Epalrestat as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Epalrestat RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, proceed as follows, using a light-resistant vessel. To 0.1 g of Epalrestat add 40 mL of methanol, dissolve the sample by warming in a water bath, and filter while hot, and cool in ice. Collect the crystals formed, dry, and perform the test.

Melting point ⟨2.60⟩ 222 – 227°C

Purity **(1)** Heavy metals ⟨1.07⟩—Proceed with 2.0 g of Epalrestat according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve about 20 mg of Epalrestat in 8 mL of *N*,*N*-dimethylformamide, and use this solution as

the sample solution. Pipet 1 mL of the sample solution, add *N*,*N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than epalrestat obtained from the sample solution is not larger than 1/5 times the peak area of epalrestat obtained from the standard solution, and the total area of the peaks other than epalrestat from the sample solution is not larger than the peak area of epalrestat from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of epalrestat, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add *N*,*N*-dimethylformamide to make exactly 10 mL. Confirm that the peak area of epalrestat obtained with 3 μL of this solution is equivalent to 7 to 13% of that obtained with 3 μL of the standard solution.

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of epalrestat are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of epalrestat is not more than 2.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 20 mg each of Epalrestat and Epalrestat RS, both previously dried, and separately dissolve in 8 mL of *N*,*N*-dimethylformamide, and add exactly 2 mL of the internal standard solution. To 2 mL each of these solutions add *N*,*N*-dimethylformamide to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of epalrestat to that of the internal standard.

Amount (mg) of epalrestat ($C_{15}H_{13}NO_3S_2$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Epalrestat RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in *N*,*N*-dimethylformamide (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 0.05 mol/L potassium dihydrogen phosphate TS add 0.05 mol/L disodium hydrogen phosphate TS so that the pH of this mixture is 6.5. To 2 volumes of this mixture add 1 volume of acetonitrile.

Flow rate: Adjust so that the retention time of epalrestat is about 12 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, epalrestat and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of epalrestat to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Epalrestat Tablets

エパルレスタット錠

Epalrestat Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of epalrestat ($C_{15}H_{13}NO_3S_2$: 319.40).

Method of preparation Prepare as directed under Tablets, with Epalrestat.

Identification (1) Powder Epalrestat Tablets. To a portion of the powder, equivalent to 50 mg of Epalrestat, add 100 mL of methanol, shake thoroughly, and filter. To 1 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm, between 290 nm and 294 nm, and between 387 nm and 391 nm.

Uniformity of dosage units <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Conduct this procedure using light-resistant vessels. To 1 tablet of Epalrestat Tablets add exactly 30 mL of *N*,*N*-dimethylformamide, shake thoroughly to completely disintegrate the tablet, and centrifuge. Pipet 1 mL of the supernatant liquid, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet *V* mL of this solution, add exactly *V′* mL of *N*,*N*-dimethylformamide so that each mL contains about 4.2 μg of epalrestat ($C_{15}H_{13}NO_3S_2$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Epalrestat RS, previously dried in vacuum at 60°C for 3 hours with silica gel as a desiccant, and dissolve in exactly 30 mL of *N*,*N*-dimethylformamide. Pipet 1 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 5 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 392 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of epalrestat ($C_{15}H_{13}NO_3S_2$)
= $M_S \times A_T/A_S \times V'/V \times 1/4$

M_S: Amount (mg) of Epalrestat RS taken

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution me-

dium, the dissolution rate in 45 minutes of Epalrestat Tablets is not less than 70%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Epalrestat Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of epalrestat ($C_{15}H_{13}NO_3S_2$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Epalrestat RS, previously dried in vacuum at 60°C for 3 hours with silica gel as a desiccant, dissolve in 10 mL of *N*,*N*-dimethylformamide, and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 398 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of epalrestat ($C_{15}H_{13}NO_3S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$

M_S: Amount (mg) of Epalrestat RS taken
C: Labeled amount (mg) of epalrestat ($C_{15}H_{13}NO_3S_2$) in 1 tablet

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Epalrestat Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of epalrestat ($C_{15}H_{13}NO_3S_2$), add 20 mL of *N*,*N*-dimethylformamide, add exactly 5 mL of the internal standard solution, shake, and centrifuge. To 2 mL of the supernatant liquid add *N*,*N*-dimethylformamide to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Epalrestat RS, previously dried in vacuum at 60°C for 3 hours with silica gel as a desiccant, dissolve in 8 mL of *N*,*N*-dimethylformamide, add exactly 2 mL of the internal standard solution, and shake. To 2 mL of this solution add *N*,*N*-dimethylformamide to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Epalrestat.

Amount (mg) of epalrestat ($C_{15}H_{13}NO_3S_2$)
$= M_S \times Q_T/Q_S \times 5/2$

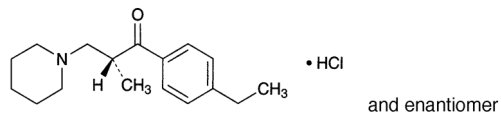
M_S: Amount (mg) of Epalrestat RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in *N*,*N*-dimethylformamide (1 in 100).

Containers and storage Containers—Tight containers.

Eperisone Hydrochloride

エペリゾン塩酸塩



and enantiomer

$C_{17}H_{25}NO.HCl$: 295.85
(2*RS*)-1-(4-Ethylphenyl)-2-methyl-3-piperidin-1-ylpropan-1-one monohydrochloride
[*56839-43-1*]

Eperisone Hydrochloride contains not less than 98.5% and not more than 101.0% of eperisone hydrochloride ($C_{17}H_{25}NO.HCl$), calculated on the anhydrous basis.

Description Eperisone Hydrochloride occurs as a white crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), and soluble in ethanol (99.5).

Melting point: about 167°C (with decomposition).

A solution of Eperisone Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Eperisone Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Eperisone Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Eperisone Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Eperisone Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Piperidine hydrochloride—Dissolve 1.0 g of Eperisone Hydrochloride in 20 mL of water, add 2.0 mL of diluted hydrochloric acid (1 in 2), 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 1.5 mL of ammonia solution (28), and use this solution as the sample solution. Separately, to 2.0 mL of a solution of piperidine hydrochloride (1 in 1000) add 18 mL of water, 2.0 mL of diluted hydrochloric acid (1 in 2), 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 1.5 mL of ammonia solution (28), and use this solution as the standard solution. To each of the sample solution and standard solution add 10 mL of a mixture of isopropylether and carbon disulfide (3:1), shake for 30 seconds, allow them to stand for 2 minutes, and compare the color of the upper layer: the color obtained from the sample solution is not more darker than that obtained from the standard solution.

(3) Related substances—Dissolve 0.1 g of Eperisone Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solu-

tion as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than eperisone from the sample solution is not larger than 1/5 times the peak area of eperisone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of methanol, 0.0375 mol/L sodium 1-decanesulfonate TS and perchloric acid (600:400:1).

Flow rate: Adjust so that the retention time of eperisone is about 17 minutes.

Time span of measurement: About 2 times as long as the retention time of eperisone.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of eperisone obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eperisone are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of eperisone is not more than 3.0%.

Water ⟨*2.48*⟩ Not more than 0.20% (0.1 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

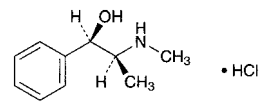
Assay Weigh accurately about 0.6 g of Eperisone Hydrochloride, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.59 mg of $C_{17}H_{25}NO.HCl$

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride

エフェドリン塩酸塩



$C_{10}H_{15}NO.HCl$: 201.69

(1*R*,2*S*)-2-Methylamino-1-phenylpropan-1-ol monohydrochloride

[*50-98-6*]

Ephedrine Hydrochloride, when dried, contains not less than 99.0% of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$).

Description Ephedrine Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in water, soluble in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in acetonitrile and in acetic anhydride.

Identification **(1)** Determine the absorption spectrum of a solution of Ephedrine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ephedrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ephedrine Hydrochloride (1 in 15) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −33.0 – −36.0° (after drying, 1 g, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Ephedrine Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 6.5.

Melting point ⟨*2.60*⟩ 218 – 222°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Ephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate—Dissolve 0.05 g of Ephedrine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 minutes: no turbidity is produced.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ephedrine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.05 g of Ephedrine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas of each peak by the automatic integration method: the total area of the peaks other than ephedrine from the sample solution is not larger than the peak area of ephedrine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 128), acetonitrile and phosphoric acid (640:360:1).

Flow rate: Adjust so that the retention time of ephedrine is about 14 minutes.

Time span of measurement: About 3 times as long as the retention time of ephedrine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of ephedrine obtained from 10 μL of this solution is equivalent to 4 to 6% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 1 mg of ephedrine hydrochloride for assay and 4 mg of atropine sulfate hydrate in 100 mL of diluted methanol (1 in 2). When the procedure is run with 10 μL of this solution under the above operating conditions, ephedrine and atropine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ephedrine is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Ephedrine Hydrochloride, previously dried, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming. Cool, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.17 mg of $C_{10}H_{15}NO.HCl$

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride Injection

エフェドリン塩酸塩注射液

Ephedrine Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$: 201.69).

Method of preparation Prepare as directed under Injections, with Ephedrine Hydrochloride.

Description Ephedrine Hydrochloride Injection is a clear, colorless liquid.

pH: 4.5 – 6.5

Identification To a volume of Ephedrine Hydrochloride Injection, equivalent to 0.05 g of Ephedrine Hydrochloride, add water to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Bacterial endotoxins ⟨*4.01*⟩ Less than 7.5 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Ephedrine Hydrochloride Injection, equivalent to about 40 mg of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$), add exactly 10 mL of the internal standard solution and water to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ephedrine to that of the internal standard.

Amount (mg) of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of ephedrine hydrochloride for assay taken

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

10% Ephedrine Hydrochloride Powder

Ephedrine Hydrochloride Powder

エフェドリン塩酸塩散 10%

10% Ephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$: 201.69).

Method of preparation

Ephedrine Hydrochloride	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make	1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

Identification To 0.5 g of 10% Ephedrine Hydrochloride Powder add 100 mL of water, shake for 20 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of 10% Ephedrine Hydrochloride Powder is not less than 85%.

Start the test with about 0.25 g of 10% Ephedrine Hydrochloride Powder, accurately weighed, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ephedrine in each solution.

Dissolution rate (%) with respect to the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$)
$= M_S/M_T \times A_T/A_S \times 9/10$

M_S: Amount (mg) of ephedrine hydrochloride for assay taken

M_T: Amount (g) of 10% Ephedrine Hydrochloride Powder taken

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrin Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ephedrine are not less than 10,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 2.0%.

Assay Weigh accurately about 0.4 g of 10% Ephedrine Hydrochloride Powder, add 150 mL of water, and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, then add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ephedrine to that of the internal standard.

Amount (mg) of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of ephedrine hydrochloride for assay taken

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Perform as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride Tablets

エフェドリン塩酸塩錠

Ephedrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$: 201.69).

Method of preparation Prepare as directed under Tablets, with Ephedrine Hydrochloride.

Identification To an amount of powdered Ephedrine Hydrochloride Tablets, equivalent to 0.05 g of Ephedrine Hydrochloride, add 100 mL of water, shake for 20 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Uniformity of dosage units <*6.02*> Perform the test accord-

ing to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ephedrine Hydrochloride Tablets add V mL of water so that each mL contains about 0.25 mg of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$), then add exactly $V/4$ mL of the internal standard solution, disperse the tablet into small particles using ultrasonic waves, then stir for a further 10 minutes in the same way. Shake this solution for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$) $= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of ephedrine hydrochloride for assay taken

Internal standard solution—A solution of etilefrine hydrochloride (1 in 2000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ephedrine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Ephedrine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of ephedrine in each solution.

Dissolution rate (%) with respect to the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$) $= M_S \times A_T/A_S \times 1/C \times 90$

M_S: Amount (mg) of ephedrine hydrochloride for assay taken

C: Labeled amount (mg) of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$) in 1 tablet

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ephedrine are not less than 10,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 2.0%.

Assay Weigh accurately not less than 20 tablets of Ephedrine Hydrochloride Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 40 mg of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$), add 150 mL of water, and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, then add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ephedrine to that of the internal standard.

Amount (mg) of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of ephedrine hydrochloride for assay taken

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

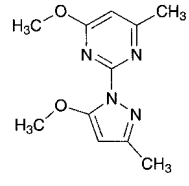
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Epirizole

Mepirizole

エピリゾール



$C_{11}H_{14}N_4O_2$: 234.25

4-Methoxy-2-(5-methoxy-3-methyl-1*H*-pyrazol-1-yl)-6-methylpyrimidine

[*18694-40-1*]

Epirizole, when dried, contains not less than 99.0% of epirizole ($C_{11}H_{14}N_4O_2$).

Description Epirizole occurs as white, crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in methanol and in acetic acid (100),

freely soluble in ethanol (95), and sparingly soluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sulfuric acid.

The pH of a solution of 1.0 g of Epirizole in 100 mL of water is between 6.0 and 7.0.

Identification **(1)** To 0.1 g of Epirizole add 0.1 g of vanillin, 5 mL of water and 2 mL of sulfuric acid, and mix with shaking for a while: a yellow precipitate is formed.

(2) Dissolve 0.1 g of Epirizole in 10 mL of water, and add 10 mL of 2,4,6-trinitrophenol TS: a yellow precipitate is produced. Collect the precipitate by filtration, wash with 50 mL of water, and dry at 105°C for 1 hour: it melts ⟨*2.60*⟩ between 163°C and 169°C.

(3) Determine the absorption spectrum of a solution of Epirizole in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point ⟨*2.60*⟩ 88 – 91°C

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Epirizole in 20 mL of water: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Add 0.5 g of Epirizole to a ground mixture of 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, mix well, transfer little by little to a platinum crucible, previously heated, and heat until the reaction is completed. After cooling, add 15 mL of dilute sulfuric acid and 5 mL of water to the residue, boil for 5 minutes, filter, wash the insoluble matter with 10 mL of water, and to the combined filtrate and washings add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: proceed with the same quantities of the same reagents as directed for the preparation of the test solution, and add 0.25 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL (not more than 0.018%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Epirizole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Epirizole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 1.0 g of Epirizole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, ethanol (95) and water (23:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Place this plate in a chamber filled with iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(6) Readily carbonizable substances ⟨*1.15*⟩—Perform the test with 0.10 g of Epirizole: the solution has no more color than Matching Fluid A.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Epirizole, previously dried, dissolve in 40 mL of acetic acid (100) and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from purple through blue-green to green.

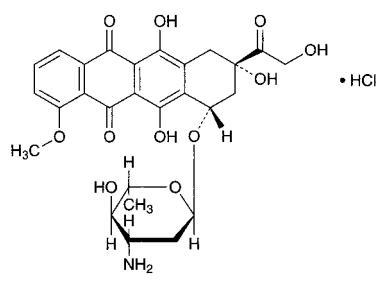
Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.43 mg of $C_{11}H_{14}N_4O_2$

Containers and storage Containers—Well-closed containers.

Epirubicin Hydrochloride

エピルビシン塩酸塩



$C_{27}H_{29}NO_{11}$.HCl: 579.98

(2*S*,4*S*)-4-(3-Amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyloxy)-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride

[*56390-09-1*]

Epirubicin Hydrochloride is the hydrochloride of a derivative of daunorubicin.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous and residual solvent-free basis. The potency of Epirubicin Hydrochloride is expressed as mass (potency) of epirubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl).

Description Epirubicin Hydrochloride occurs as a pale yellowish red to brownish red powder.

It is soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Epirubicin Hydrochloride in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Epirubicin Hydrochloride and Epirubicin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +310 – +340° (10 mg calculated on the anhydrous and residual solvent-free basis, meth-

anol, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 10 mg of Epirubicin Hydrochloride in 2 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 50 mg of Epirubicin Hydrochloride in 5 mL of water: the solution is clear and dark red.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Epirubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, determine each peak area by the automatic integration method, and calculate the total amount of the peaks other than epirubicin and 2-naphthalenesulfonic acid by the area percentage method: not more than 5.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of epirubicin, beginning after the solvent peak.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of epirubicin obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

(4) Residual solvents ⟨*2.46*⟩—Weigh accurately about 0.3 g of Epirubicin Hydrochloride, add exactly 0.6 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 6 mL, and use this solution as the sample solution. Separately, pipet 1 mL of methanol, add *N*,*N*-dimethylformamide to make exactly 25 mL, and use this solution as methanol standard stock solution. Take exactly 125 μL of acetone, 30 μL of ethanol (99.5), 32 μL of 1-propanol and 17 μL of the methanol standard stock solution, add exactly 10 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following condition, and calculate the ratios of the peak areas of acetone, ethanol, 1-propanol and methanol to that of the internal standard, Q_{Ta} and Q_{Sa}, Q_{Tb} and Q_{Sb}, Q_{Tc} and Q_{Sc}, and Q_{Td} and Q_{Sd}, respectively. Calculate the amounts of acetone, ethanol, 1-propanol and methanol by the following equations: the amounts of acetone, ethanol, 1-propanol and methanol are not more than 1.5%, not more than 0.5%, not more than 0.5% and not more than 0.1%, respectively.

Amount (%) of acetone = $1/M_T \times Q_{Ta}/Q_{Sa} \times 593$

Amount (%) of ethanol = $1/M_T \times Q_{Tb}/Q_{Sb} \times 142$

Amount (%) of 1-propanol = $1/M_T \times Q_{Tc}/Q_{Sc} \times 154$

Amount (%) of methanol = $1/M_T \times Q_{Td}/Q_{Sd} \times 2.23$

M_T: Amount (mg) of Epirubicin Hydrochloride taken

Internal standard solution—A solution of 1,4-dioxane in *N*,*N*-dimethylformamide (1 in 100).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter and 30 m in length, coated with polyethylene glycol for gas-chromatography 1 μm in thickness.

Column temperature: 40°C for 11 minutes after injection of the sample, then rise to 90°C at a rate of 10°C per minute. If necessary, rise to 130°C at a rate of 50°C per minute and maintain the temperature for 30 minutes.

Injection port temperature: A constant temperature of about 120°C.

Detector temperature: A constant temperature of about 150°C.

Carrier gas: Herium.

Flow rate: Adjust so that the retention time of the internal standard is about 8 minutes.

Split ratio: 1:15.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone, methanol, ethanol, 1-propanol and the internal standard are eluted in this order with the resolution between the peaks of acetone and the internal standard being not less than 30.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of acetone, methanol, ethanol and 1-propanol are not more than 4.0%, respectively.

Water ⟨*2.48*⟩ Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (0.1 g).

Assay Weigh accurately an amount of Epirubicin Hydrochloride and Epirubicin Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in the internal standard solution to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of epirubicin to that of the internal standard.

Amount [μg (potency)] of epirubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Epirubicin Hydrochloride RS taken

Internal standard solution—A solution of sodium 2-naphthalene sulfonate in a mixture of water, acetonitrile, methanol and phosphoric acid (540:290:170:1) (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (6 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in a mixture of water, acetonitrile, methanol and phosphoric acid (540:290:170:1) to make 1000 mL.

Flow rate: Adjust so that the retention time of epirubicin is about 9.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and epirubicin are eluted in this order with the resolution between these peaks being not less than 20.

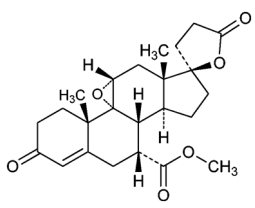
System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epirubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At a temperature between 0°C and 5°C.

Eplerenone

エプレレノン



$C_{24}H_{30}O_6$: 414.49

9,11α-Epoxy-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone

[*107724-20-9*]

Eplerenone contains not less than 98.0% and not more than 102.0% of eplerenone ($C_{24}H_{30}O_6$), calculated on the dried basis.

Description Eplerenone occurs as a white crystalline powder.

It is freely soluble in acetonitrile, sparingly soluble in methanol, and very slightly soluble in water and in ethanol (99.5).

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Eplerenone in methanol (1 in 77,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Eplerenone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Eplerenone as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Eplerenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −14.0 – −16.0° (0.25 g calculated on the dried basis, acetonitrile, 25 mL, 100 mm).

Purity **(1)** Heavy metals—Take 1.0 g of Eplerenone in a crucible, wet the sample with a suitable amount of sulfuric acid, cover loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid, and 5 drops of sulfuric acid to the carbonized residue, and heat gently until white fumes are no longer evolved. Then, incinerate by ignition at 500 – 600°C. After cooling, add 4 mL of 6 mol/L hydrochloric acid TS, cover the crucible, warm on a water bath for 15 minutes, then remove the cover from the crucible, and slowly evaporate to dryness on a water bath. Wet the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. After cooling, add ammonia TS until the solution shows alkalinity to litmus paper, add 15 mL of water, and adjust to pH 3.0 – 4.0 with dilute acetic acid. Filter, if necessary, wash the crucible and filter paper with 10 mL of water, put the filtrate and the washings in a Nessler tube, add water to make 40 mL, and use this solution as the sample solution. Separately, take 2.0 mL of Standard Lead Solution in a Nessler tube, and add water to make 25 mL. Adjust to pH 3.0 – 4.0 of this solution with dilute acetic acid or ammonia TS, add water to make 40 mL, and use this solution as the control solution. To the sample solution and the control solution add 2 mL of acetate buffer solution (pH 3.5) and 1.2 mL of thioacetamide-alkaline glycerin TS, then add water to make 50 mL, allow them to stand for 2 minutes, and observe vertically against a white background: the solution obtained from the sample solution is not more colored than that obtained from the control solution (not more than 20 ppm).

(2) Related substances—Dissolve 25 mg of Eplerenone in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 0.58, about 0.85, about 0.90, about 1.2 and about 1.6 to eplerenone, obtained from the sample solution is respectively not larger than 1/5, 3/10, 3/10, 3/10 and 3/10 times the peak area of eplerenone obtained from the standard solution, and the area of the peak other than eplerenone and the peak mentioned above from the sample solution is not larger than 7/50 times the peak area of eplerenone from the standard solution. Furthermore, the total area of the peaks other than eplerenone from the sample solution is not larger than 1.2 times the peak area of eplerenone from the standard solution. For the area of the peak, having the relative retention time of about 0.85 to eplerenon, multiply the relative response factor 0.6.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of eplerenone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 10 mL. Confirm that the peak area of eplerenone obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eplerenone are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of eplerenone is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 4 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Eplerenone and Eplerenone RS (separately determine the loss on drying ⟨2.41⟩ under the same conditions as Eplerenone), separately dissolve in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of eplerenone in each solution.

Amount (mg) of eplerenone ($C_{24}H_{30}O_6$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Eplerenone RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 580 mL of this solution add 360 mL of acetonitrile for liquid chromatography and 60 mL of methanol.

Flow rate: Adjust so that the retention time of eplerenone is about 12 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eplerenone are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of eplerenone is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Eplerenone Tablets

エプレレノン錠

Eplerenone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of eplerenone ($C_{24}H_{30}O_6$: 414.49).

Method of preparation Prepare as directed under Tablets, with Eplerenone.

Identification Determine the absorption spectrum of the sample solution obtained in the Uniformity of dosage units as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩: it exhibits a maximum between 240 nm and 244 nm.

Uniformity of dosage units ⟨6.02⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Eplerenone Tablets add a suitable amount of a mixture of acetonitrile and water (3:2), shake, disintegrate the tablet with the aid of ultrasonic waves, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and centrifuge. Take exactly V mL of the supernatant liquid, add a mixture of acetonitrile and water (3:2) to make exactly V' mL so that each mL contains about 25 μg of eplerenone ($C_{24}H_{30}O_6$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Eplerenone RS (separately determine the loss on drying ⟨2.41⟩ under the same conditions as Eplerenone), and dissolve in a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 243 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩.

Amount (mg) of eplerenone ($C_{24}H_{30}O_6$)
= $M_S \times A_T/A_S \times V'/V \times 1/10$

M_S: Amount (mg) of Eplerenone RS taken, calculated on the dried basis

Dissolution ⟨6.10⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Eplerenone Tablets is not less than 75%.

Start the test with 1 tablet of Eplerenone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 11 μg of eplerenone ($C_{24}H_{30}O_6$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Eplerenone RS (separately determine the loss on drying ⟨2.41⟩ under the same conditions as Eplerenone), dissolve in 5 mL of acetonitrile, and add the dissolution medium to make exactly 500 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 243 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩ using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of eplerenone ($C_{24}H_{30}O_6$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Eplerenone RS taken, calculated on the dried basis

C: Labeled amount (mg) of eplerenone ($C_{24}H_{30}O_6$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Eplerenone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of eplerenone ($C_{24}H_{30}O_6$), add a suitable amount of a mixture of acetonitrile and water (3:2), agitate to disperse the particles with the aid of ultrasonic waves, and add a mixture of acetonitrile and water (3:2) to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Eplerenone RS (separately determine the loss on drying ⟨2.41⟩ under the same conditions as Eplerenone), dissolve in a mixture of acetonitrile and water (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the peak

areas, A_T and A_S, of eplerenone in each solution.

$$\text{Amount (mg) of eplerenone } (C_{24}H_{30}O_6) = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of Eplerenone RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 243 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 550 mL of this solution add 360 mL of methanol and 90 mL of acetonitrile.

Flow rate: Adjust so that the retention time of eplerenone is about 12 minutes.

System suitability—

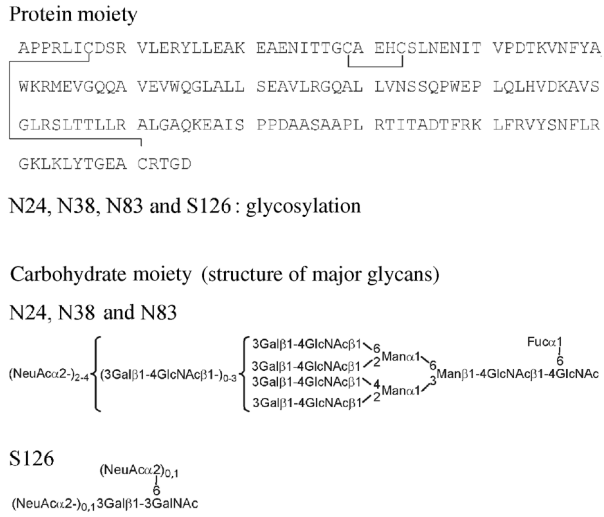
System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eplerenone are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of eplerenone is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Epoetin Alfa (Genetical Recombination)

エポエチン　アルファ（遺伝子組換え）



$C_{809}H_{1301}N_{229}O_{240}S_5$: 18235.70 (Protein moiety)
[*113427-24-0*]

Epoetin Alfa (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human erythropoietin produced in Chinese hamster ovary cells. It is a glycoprotein (molecular mass: ca. 37,000 to 42,000) consisting of 165 amino acid residues. It has stimulatory effects for the differentiation and proliferation of erythroid precursor.

It contains not less than 1.1 mg and not more than 1.5 mg of protein per mL, and not less than 1.5×10^5 units per mg of protein.

Description Epoetin Alfa (Genetical Recombination) occurs as a clear and colorless liquid.

Identification **(1)** Dilute a suitable volume of Epoetin Alfa (Genetical Recombination) and Epoetin Alfa RS with water. To 3 volume of these solutions add 1 volume each of buffer solution for epoetin alfa sample, heat at 100°C for 5 minutes, and use these solutions as the sample solution and the standard solution, respectively. Transfer a volume of the sample solution and the standard solution, equivalent to 0.7 μg of protein, into each sample well of the polyacrylamide gel for epoetin alfa, and start the SDS-polyacrylamide gel electrophoresis using a vertical discontinuous buffer solution system. After the electrophoresis, immerse the gel, a polyvinylidene fluoride membrane and a filter paper in the blotting TS. Set them on a semi-dry blotting apparatus, and transcribe for about 1 hour with a constant electric current of 0.7 – 0.9 mA/cm^2 depending on the dimension of the filter paper. Then, immerse the polyvinylidene fluoride membrane in the blocking TS for epoetin alfa for more than 1 hour while shaking, remove the blocking TS for epoetin alfa and add the primary antibody TS, then shake for a night or allow to stand at 4°C for 3 nights. Remove the primary antibody TS, wash the membrane with phosphate-buffered sodium chloride TS, add the secondary antibody TS, and shake for more than 1 hour. Remove the secondary antibody TS, wash the membrane with phosphate-buffered sodium

chloride TS, add the avidin-biotin TS, and shake for more than 1 hour. Remove the avidin-biotin TS, wash the membrane with phosphate-buffered sodium chloride TS, and add the substrate TS for epoetin alfa for developing the color image: the main stained bands obtained from the sample solution appear as similar migrating image as those obtained from the standard solution.

(2) Evaporate to dryness under reduced pressure a volume of Epoetin Alfa (Genetical Recombination) and Epoetin Alfa RS, equivalent to about 35 μg of protein, and dissolve these residues in 100 μL of 0.1 mol/L tris buffer solution (pH 7.3). To these solutions add 5 μL of trypsin TS for epoetin alfa, warm at 37°C for 6 hours, then cool in ice, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 45 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the chromatograms obtained from these solutions: both chromatograms show the similar peaks at the corresponding retention time.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase A: A mixture of water and trifluoroacetic acid (5000:3).

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (4000:1000:3).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	98	2
5 – 95	98 → 35	2 → 65

Flow rate: 0.75 mL per minute.

System suitability—

System performance: When the procedure is run with 45 μL of the standard solution under the above conditions, the chromatogram shows the similar pattern with the chromatogram of Epoetin Alfa RS obtained in the Peptide mapping.

Oligosaccharide profile Being specified separately when the drug is granted approval based on the Law.

Sialic acid content To an exact volume of Epoetin Alfa (Genetical Recombination), equivalent to about 1 nmol of protein, add water to make exactly 45 μL. Add exactly 5 μL of sodium hydroxide TS, allow to stand in ice water for 90 minutes, and add exactly 5 μL of dilute acetic acid. Add exactly 45 μL of water and exactly 100 μL of a mixture of water and acetic acid (100) (27:8), and warm at 80°C for 210 minutes. After cooling, add exactly 200 μL of the fluorescence TS, and warm at 60°C for 2 hours avoiding exposure to light. After cooling, add exactly 200 μL of sodium hydroxide TS, and use this solution as the sample solution. Separately, just before starting the test, to exactly 250 μL of 0.4 mmol/L *N*-acetylneuraminic acid TS add exactly 20 μL of 0.1 mmol/L *N*-glycolylneuraminic acid TS and exactly 180 μL of water. Proceed with exactly 45 μL of this solution in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the peak areas of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid, A_{T1} and A_{T2}, obtained from the sample solution, and the peak areas of those, A_{S1} and A_{S2}, obtained from the standard solution. Calculate the content of sialic acid in Epoetin Alfa (Genetical Recombination) by the following equation: between 10 mol/mol and 12 mol/mol.

$$\text{Content (mol/mol) of sialic acid} = (A_{T1}/A_{S1} \times 10 + A_{T2}/A_{S2} \times 1/5)/a$$

a: Number (nmol) of moles of Epoetin Alfa (Genetical Recombination)

where, molar concentration (mmol/L) of Epoetin Alfa (Genetical Recombination) is calculated by the following equation, using the absorbance A at 280 nm obtained in the Assay (1).

$$\text{Molar concentration (mmol/L) of Epoetin Alfa (Genetical Recombination)} = A \times 10^3/22{,}430$$

22,430: Molar absorbance coefficient ε

Operating conditions—

Detector: A fluorophotometer (excitation wavelength: 373 nm, fluorescence wavelength: 448 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile and methanol (84:9:7).

Mobile phase B: A mixture of water and methanol (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	100	0
20 – 20.1	100 → 0	0 → 100
20.1 – 27	0	100

Flow rate: 0.6 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, *N*-glycolylneuraminic acid and *N*-acetylneuraminic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of *N*-glycolylneuraminic acid and *N*-acetylneuraminic acid are not more than 2.0%, respectively.

Molecular mass Use the sample solution obtained in the Identification (1) as the sample solution. Separately, to 20 μL of molecular mass standard stock solution add 6.7 μL of the buffer solution for epoetin alfa sample, heat at 100°C for 5 minutes, and use this solution as the molecular mass standard solution. Transfer a volume of the sample solution, equivalent to 3.5 μg of protein and the total volume of the molecular mass standard solution into each sample well of

the vertical discontinuous buffer solution system SDS-polyacrylamide gel, composed with resolving and stacking gels, and perform the electrophoresis. After the electrophoresis, immerse the gel in a solution of Coomassie brilliant blue R-250, containing 1.25 g in a mixture of 450 mL of methanol, 100 mL of acetic acid (100) and sufficient amount of water making up to 1000 mL. Determine the relative mobilities of the stained bands of egg albumin (molecular mass: about 45,000), carbonic anhydrase (molecular mass: about 31,000), soybean trypsin inhibitor (molecular mass: about 21,500) and lysozyme (molecular mass: 14,400), and prepare a calibration curve by linear regression against the logarithm of the molecular masses. Determine the relative mobility of the center of the main band obtained from the sample solution, and calculate the molecular mass of Epoetin Alfa (Genetical Recombination) from the calibration curve: it is between 37,000 and 42,000.

pH <2.54> 5.7 – 6.7

Purity **(1)** Multimers—Perform the test with a volume of Epoetin Alfa (Genetical Recombination), equivalent to 50 μg of protein, as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the total amount of the peaks other than epoetin alfa is not more than 2%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 91 mg of disodium hydrogen phosphate dodecahydrate, 0.27 g of sodium dihydrogen phosphate dihydrate and 8.77 g of sodium chloride in water to make 1000 mL.

Flow rate: Adjust so that the retention time of epoetin alfa is about 16 minutes.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column until the elution of epoetin alfa is finished.

System suitability—

Test for required detectability: To 1 volume of Epoetin Alfa (Genetical Recombination) add 49 volumes of the mobile phase, and use this solution as the solution for system suitability test. Confirm that the peak area of epoetin alfa obtained with a volume, equivalent to 1 μg of protein, of the solution for system suitability test is equivalent to 1.5 to 2.5% of that obtained with the same volume of Epoetin Alfa (Genetical Recombination).

System performance: Dissolve 40 mg of bovine serum albumin for gel filtration molecular mass marker and 20 mg of chymotrypsinogen for gel filtration molecular mass marker in 100 mL of the mobile phase. When the procedure is run with 50 μL of this solution under the above operating conditions, bovine serum albumin and chymotrypsinogen are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with a volume of Epoetin Alfa (Genetical Recombination), equivalent to 50 μg of protein, under the above operating conditions, the relative standard deviation of the area of the principal peak of epoetin alfa is not more than 2.0%.

(2) Host cell proteins—Being specified separately when the drug is granted approval based on the Law.

(3) DNA—Being specified separately when the drug is granted approval based on the Law.

Assay **(1)** Protein content—Take a suitable amount of Epoetin Alfa (Genetical Recombination), dilute with phosphate buffer solution for epoetin alfa, if necessary, so that each mL contains 0.5 – 0.8 mg protein and use the solution as the sample solution. Determine the absorbance, A, at 280 nm of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the phosphate buffer solution for epoetin alfa as the blank.

Amount (mg) of protein in 1 mL of Epoetin Alfa (Genetical Recombination)
$= A \times d \times 0.909$

d: Dilution factor for the sample solution
0.909: Reciprocal number of absorption coefficient ($E^{0.1\%}_{1\,cm}$) of epoetin alfa protein

(2) Specific activity

(i) Animals: Select healthy 6 to 8 weeks female mice (B6D2F1, etc.). Keep the mice for not less than a week before use, providing an appropriate uniform diet and water.

(ii) Standard solutions: To Epoetin Alfa RS add the bovine serum albumin-saline solution so that each mL contains exactly 10 – 40 units, and designate this solution as the high-dose standard solution, S_H. Dilute S_H exactly 4 times with the bovine serum albumin-saline solution, and designate this solution as the low-dose standard solution, S_L.

(iii) Sample solutions: To Epoetin Alfa (Genetical Recombination) add the bovine serum albumin-saline solution to make two sample solutions, the high-dose sample solution, T_H, which contains the Units per mL equivalent to S_H and the low-dose sample solution, T_L, which contains the Units per mL equivalent to S_L.

(iv) Procedure: Divide the animals into 4 equal groups of not less than 5 animals each. On the 1st, 2nd and 3rd days, inject exactly 0.2 mL each of the standard solutions and the sample solutions into each animal subcutaneously as indicated in the following design:

First group	S_H	Third group	T_H
Second group	S_L	Fourth group	T_L

On the 4th day, take a sufficient blood sample to perform the test from each animal. To 10 mL of the dilution fluid for particle counter add exactly 20 μL of the blood sample, mix, add 100 μL of the appropriate hemolysis agent, stir for 5 minutes, and determine the count of particles derived from hemolytic-resistant erythroid cells.

(v) Calculation: Logarithmic converted counts of the fine particles obtained with S_H, S_L, T_H and T_L in (iv) are symbolized as y_1, y_2, y_3 and y_4, respectively. Sum up individual y_1, y_2, y_3 and y_4 to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

Specific activity (unit/mg protein) of Epoetin Alfa (Genetical Recombination)
= activity (unit/mL) of Epoetin Alfa (Genetical Recombination)/C

Activity (unit/mL) of Epoetin Alfa (Genetical Recombination)
= antilog $M \times$ unit in 1 mL of $S_H \times d$

$M = \log 4 \times Y_a/Y_b$
$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$
$Y_b = Y_1 - Y_2 + Y_3 - Y_4$

d: Dilution factor for T_H
C: Concentration (mg/mL) of protein obtained in Assay (1)

F' computed by the following equation should be smaller than F shown in the table against n with which s^2 is calculated. Calculate L (p = 0.95) by use of the following equation: L should be not more than 0.3. If F' exceeds F, or if L exceeds 0.3, repeat the test, arranging the assay conditions.

$$F' = (Y_1 - Y_2 - Y_3 + Y_4)^2/4fs^2$$

f: Number of animals per group, which should be the same for each group and not less than 5.

$$s^2 = (\Sigma y^2 - Y/f)/n$$

Σy^2: The sum of the squares of each y_1, y_2, y_3 and y_4.
$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$
$n = 4\,(f - 1)$

$$L = 2\sqrt{(C - 1)\{CM^2 + (\log 4)^2\}}$$

$$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$$

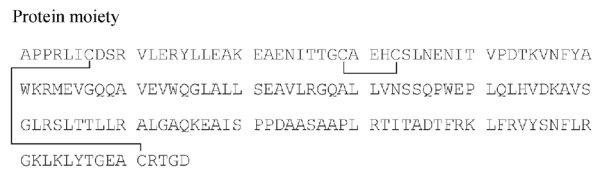
$F\,(= t^2)$ values against n

n	$t^2 = F$	n	$t^2 = F$	n	$t^2 = F$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.
Storage—Not exceeding −70°C.

Epoetin Beta (Genetical Recombination)

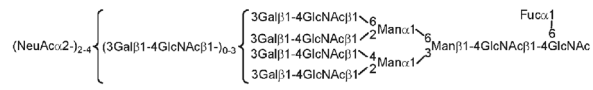
エポエチン　ベータ（遺伝子組換え）



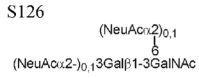
N24, N38, N83 and S126: glycosylation

Carbohydrate moiety (structure of major glycans)

N24, N38 and N83



S126



$C_{809}H_{1301}N_{229}O_{240}S_5$: 18235.70 (Protein moiety)
[*122312-54-3*]

Epoetin Beta (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human erythropoietin produced in Chinese hamster ovary cells. It is a glycoprotein (molecular mass: ca. 30,000) consisting of 165 amino acid residues. It has stimulatory effects for the differentiation and proliferation of erythroid progenitor cell.

It contains not less than 0.5 mg and not more than 1.5 mg of protein per mL, and not less than 1.5×10^5 units per mg of protein.

Description Epoetin Beta (Genetical Recombination) occurs as a clear and colorless liquid.

Identification **(1)** Use Epoetin Beta (Genetical Recombination) and Epoetin Beta RS as the sample solution and the standard solution, respectively. When perform a capillary electrophoresis with the sample solution and standard solution according to the following conditions, the mobility of each peak obtained from both solutions is the same and their migrating images are similar each other.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A silica capillary tube 50 μm in inside diameter and about 50 cm in length, chemically coated inner surface with amino groups (about 40 cm in effective length).

Electrolyte solution: Dissolve 32.8 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 4.5 with a solution, prepared by dissolving 75.2 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 19 volumes of this solution add 1 volume of ethanol (99.5).

Running temperature: A constant temperature of about 20°C.

Running conditions: Migration current (a constant current of about 45 μA), migration time (30 minutes).

Injection of sample and standard solutions: 5 seconds (pressurization: 0.5 psi).

Time span of measurement: From 10 minutes to 30

minutes after injection (excluding the peak of solvent origin).

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, more than 4 major peaks of epoetin beta are detected, and the resolution between the first and second eluted major peaks is not less than 0.8.

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the migration time of the first eluted major peak is not more than 2.0%.

(2) Desalt a volume each of Epoetin Beta (Genetical Recombination) and Epoetin Beta RS, equivalent to 600 μg of protein, by a suitable method, and term them as the desalted sample and the desalted reference standard, respectively. Dissolve the desalted sample and the desalted reference standard in 600 μL each of a solution, prepared by dissolving 2.3 g of *N*-ethylmorpholine in 100 mL of water and adjusting to pH 8.0 with acetic acid (100), and use these solutions as the desalted sample solution and the desalted reference standard solution, respectively. To 500 μL each of the desalted sample solution and the desalted reference standard solution add 3.3 μL of triethylamine for epoetin beta and 1.5 μL of 2-mercaptoethanol for epoetin beta, and react at 37°C for 1 hour. After cooling, add 5.5 μL of 4-vinylpyridine to them, and react at 25°C for 1 hour. To these solutions add 50 μL of diluted trifluoroacetic acid for epoetin beta (1 in 10) to stop the reaction, remove the reagents by a suitable method, and use the substances so obtained as the pyridylethylated sample and the pyridylethylated reference substance, respectively. Dissolve the pyridylethylated sample and the pyridylethylated reference substance separately in 500 μL of sodium hydrogen carbonate solution (21 in 2500). To 400 μL each of these solutions add 16 μL of a solution of lysyl endopeptidase in sodium hydrogen carbonate solution (21 in 2500) (1 in 50,000), and react at 37°C for 24 hours. While this reaction, additional two 16-μL portions of a solution of lysyl endopeptidase in sodium hydrogen carbonate (21 in 2500) (1 in 50,000) are added at 4 hours and 20 hours after starting the reaction. Then, stop the reaction by adding 100 μL of diluted trifluoroacetic acid for epoetin beta (1 in 10), and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from these solutions: both chromatograms show the similar peaks at the corresponding retention times.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water and trifluoroacetic acid for epoetin beta (1000:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water, and trifluoroacetic acid for epoetin beta (900:100:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	90	10
10 – 30	90 → 80	10 → 20
30 – 50	80	20
50 – 130	80 → 40	20 → 60
130 – 140	40 → 10	60 → 90
140 – 150	10	90

Flow rate: Adjust so that the retention time of the first peak, which appears after the solvent peak, is about 17 minutes.

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, 9 major peptide peaks are appeared after the solvent peak, and the resolution between the peaks eluted at the fifth and the sixth is not less than 3.

Sialic acid content To exactly 100 μL of Epoetin Beta (Genetical Recombination) add 1 mL of resorcinol-copper (II) sulfate TS, and heat on a water bath for 30 minutes. After ice-cooling, add 2 mL of a mixture of *n*-butyl acetate and 1-butanol (4:1), shake vigorously, and use the upper layer as the sample solution. Separately, dissolve *N*-acetylneuraminic acid in water to make three solutions, containing 0.1 mg, 0.2 mg and 0.3 mg of *N*-acetylneuraminic acid in each mL, and use these solutions as the standard stock solution (1), the standard stock solution (2) and the standard stock solution (3), respectively. Pipet 100 μL each of these standard stock solutions, add 1 mL of resorcinol-copper (II) sulfate TS to them, then proceed in the same way as for the sample solution, and use these solutions so obtained as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Determine the absorbances of the sample solution and the standard solutions (1), (2) and (3) at 625 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Calculate the amount of sialic acid (mg/mL) in the sample solution, by using the calibration curve obtained from the standard solutions, and calculate the amount of sialic acid in Epoetin Beta (Genetical Recombination) by the following equation: between 10 mol/mol and 13 mol/mol.

$$\text{Amount of sialic acid (mol/mol of epoetin beta protein)} = A/C \times 18{,}236/309.27$$

A: Amount (mg/mL) of sialic acid in the sample solution
C: Amount (mg/mL) of protein in Epoetin Beta (Genetical Recombination)
18,236: Molecular mass of protein moiety of epoetin beta
309.27: Molecular mass of *N*-acetylneuraminic acid

Oligosaccharide profile Being specified separately when the drug is granted approval based on the Law.

pH <*2.54*> 7.0 – 8.0

Purity (1) Related substances—Perform the test with 20 μL of Epoetin Beta (Genetical Recombination) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of these peaks other than the solvent peak by the area percentage method: the total area of the peaks other than epoetin beta is not more than 1.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 214 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.6 g of sodium dihydrogen phosphate dihydrate and 16.1 g of sodium sulfate decahydrate in water to make 1000 mL, and adjust to pH 6.8 with a solution, prepared by dissolving 16.1 g of sodium sulfate decahydrate in 0.01 mol/L sodium hydroxide TS to make 1000 mL.

Flow rate: Adjust so that the retention time of epoetin beta is about 18 minutes.

Time span of measurement: About 2 times as long as the retention time of epoetin beta.

System suitability—

Test for required detectability: When the procedure is run with 20 μL of diluted Epoetin Beta RS with water containing 0.05 vol% polysorbate 20 for epoetin beta (1 in 1000) under the above conditions, the peak of epoetin beta is detectable.

System performance: When the procedure is run with Epoetin Beta RS under the above conditions, the number of theoretical plates of the peak of epoetin beta is not less than 600.

System repeatability: When the test is repeated 6 times with 20 μL of Epoetin Beta RS under the above operating conditions, the relative standard deviation of the peak area of epoetin beta is not more than 1.0%.

(2) Host cell proteins—Being specified separately when the drug is granted approval based on the Law.

(3) DNA—Being specified separately when the drug is granted approval based on the Law.

Assay (1) Protein content—Use Epoetin Beta (Genetical Recombination) and Epoetin Beta RS as the sample solution and the standard solution, respectively. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the total area, A_T and A_S, of the main peak and the sub-peak of epoetin beta in each solution.

Amount (mg) of protein in 1 mL of Epoetin Beta (Genetical Recombination)
$= C_S \times A_T/A_S$

C_S: Protein concentration (mg/mL) of Epoetin Beta RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with butylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid for epoetin beta (400:100:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water and trifluoroacetic acid for epoetin beta (400:100:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 18	65 → 50	35 → 50
18 – 33	50 → 0	50 → 100
33 – 43	0	100

Flow rate: Adjust so that the retention time of the main peak of epoetin beta is about 22 minutes.

System suitability—

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the main peak and the sub-peak of epoetin beta are eluted in this order, and the number of theoretical plates of the main peak is not less than 600.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the total area of the main peak and the sub-peak of epoetin beta is not more than 4.0%.

(2) Specific activity—To Epoetin Beta (Genetical Recombination) add 0.1 w/v% bovine serum albumin-sodium chloride-phosphate buffer solution to make three solutions so that each mL contains epoetin beta equivalent to 5, 10 and 20 units (estimate), and use these solutions as the sample solutions (1), (2) and (3), respectively. Separately, to Epoetin Beta RS add 0.1 w/v% bovine serum albumin-sodium chloride-phosphate buffer solution to make three solutions so that each mL contains epoetin beta equivalent to 5, 10 and 20 units, and use these solutions as the standard solutions (1), (2) and (3), respectively. Divide ICR strain mice into 6 equal groups of not less than 5 mice. Inject exactly 0.2 mL each of the sample solutions and the standard solutions to ICR strain mice of each group subcutaneously on the 1st, 2nd and 3rd days. On the 4th day, collect the blood from the mice, put 20 μL each of the collected blood in 9.94 mL of blood dilution liquid, mix, and use these mixtures as the dilute blood solution. To each of the dilute blood solution add 100 μL of a hemolytic agent, mix gently to hemolyze, and count the particles of hemolytic agent-resistant red cell by using a particle counter.

Determine the potency ratio (P_r) of the sample solution to the standard solution, and calculate the unit per mg protein of Epoetin Beta (Genetical Recombination) by the following equation.

$$P_r = 10^M$$
$$M = 4/3 \times i \times T_a/T_b$$
$$i = \log 2$$
$$T_a = -S_1 - S_2 - S_3 + U_1 + U_2 + U_3$$
$$T_b = -S_1 + S_3 - U_1 + U_3$$

U_1: Sum of the responses obtained from the sample solution (1)

U_2: Sum of the responses obtained from the sample solution (2)

U_3: Sum of the responses obtained from the sample solution (3)

S_1: Sum of the responses obtained from the standard solution (1)

S_2: Sum of the responses obtained from the standard solution (2)

S_3: Sum of the responses obtained from the standard solution (3)

Specific activity (unit/mg of protein) of Epoetin Beta (Genetical Recombination)
$= S \times P_r \times D_T/D_S/C$

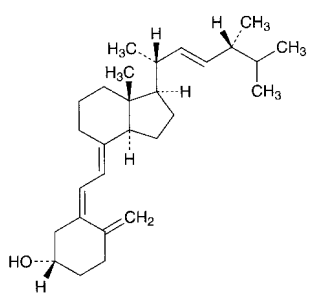
S: Potency (unit/mL) of Epoetin Beta RS
D_T: Dilution factor for the sample solution (3)
D_S: Dilution factor for the standard solution (3)
C: Protein amount (mg/mL) of Epoetin Beta (Genetical Recombination)

Containers and storage Containers—Tight containers.
Storage—Not exceeding −20°C.

Ergocalciferol

Calciferol
Vitamin D_2

エルゴカルシフェロール



$C_{28}H_{44}O$: 396.65
(3*S*,5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol
[*50-14-6*]

Ergocalciferol contains not less than 97.0% and not more than 103.0% of ergocalciferol ($C_{28}H_{44}O$).

Description Ergocalciferol occurs as white crystals. It is odorless, or has a faint, characteristic odor.

It is freely soluble in ethanol (95), in diethyl ether and in chloroform, sparingly soluble in isooctane, and practically insoluble in water.

It is affected by air and by light.

Melting point: 115 – 118°C Transfer Ergocalciferol to a capillary tube, and dry for 3 hours in a desiccator (in vacuum at a pressure not exceeding 2.67 kPa). Immediately fire-seal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10°C below the expected melting point, and heat at a rate of rise of about 3°C per minute, and read the melting point.

Identification **(1)** Dissolve 0.5 mg of Ergocalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared absorption spectrum of Ergocalciferol as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ergocalciferol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (265 nm): 445 – 485 (10 mg, ethanol (95), 100 mL).

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +102 – +107° (0.3 g, ethanol (95), 20 mL, 100 mm). Prepare the solution of Ergocalciferol within 30 minutes after the container has been opened, and determine the rotation within 30 minutes after the solution has been prepared.

Purity Ergosterol—Dissolve 10 mg of Ergocalciferol in 2.0 mL of diluted ethanol (9 in 10), add a solution of 20 mg of digitonin in 2.0 mL of diluted ethanol (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay Weigh accurately about 30 mg each of Ergocalciferol and Ergocalciferol RS, and dissolve each in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 to 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ergocalciferol to that of the internal standard. Perform the procedure rapidly avoiding contact with air or other oxidizing agents and using light-resistant containers.

Amount (mg) of ergocalciferol ($C_{28}H_{44}O$) $= M_S \times Q_T/Q_S$

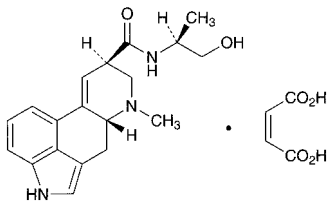
M_S: Amount (mg) of Ergocalciferol RS taken

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with a silica gel for liquid chromatography (10 μm particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: A mixture of hexane and *n*-amylalcohol (997:3).
Flow rate: Adjust so that the retention time of ergocalciferol is about 25 minutes.
System suitability—
System performance: Dissolve 15 mg of Ergocalciferol RS in 25 mL of isooctane. Transfer this solution to a flask, heat in an oil bath under a reflux condenser for 2 hours, and cool immediately to room temperature. Transfer the solution to a quartz test tube, and irradiate with a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To 10 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the ratios of the retention time of previtamin D_2, trans-vitamin D_2 and tachysterol$_2$ to that of ergocalciferol are about 0.5, about 0.6 and about 1.1, respectively, and the resolution between previtamin D_2 and trans-vitamin D_2 is not less than 0.7, and that between ergocalciferol and tachysterol$_2$ is not less than 1.0.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ergocalciferol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

Ergometrine Maleate

エルゴメトリンマレイン酸塩



$C_{19}H_{23}N_3O_2.C_4H_4O_4$: 441.48
(8*S*)-*N*-[(1*S*)-2-Hydroxy-1-methylethyl]-6-methyl-9,10-didehydroergoline-8-carboxamide monomaleate
[*129-51-1*]

Ergometrine Maleate, when dried, contains not less than 98.0% of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$).

Description Ergometrine Maleate occurs as a white to pale yellow crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 185°C (with decomposition).

It gradually changes to yellow in color on exposure to light.

Identification **(1)** Prepare a solution of Ergometrine Maleate (1 in 50): the solution shows a blue fluorescence.

(2) Dissolve 1 mg of Ergometrine Maleate in 5 mL of water. To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, shake, and allow to stand for 5 to 10 minutes: a deep blue color develops.

(3) To 5 mL of a solution of Ergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the solution disappears immediately.

Optical rotation <*2.49*> $[\alpha]_D^{20}$ +48 – +57° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water. The pH of the solution is between 3.0 and 5.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Ergotamine and ergotoxine—To 0.02 g of Ergometrine Maleate add 2 mL of a solution of sodium hydroxide (1 in 10), and heat to boiling: the gas evolved does not change moistened red litmus paper to blue.

(3) Related substances—Dissolve 5.0 mg each of Ergometrine Maleate and Ergometrine Maleate RS in 1.0 mL of methanol, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate, prepared with silica gel for thin-layer chromatography and dilute sodium hydroxide TS. Develop the plate with a mixture of chloroform and methanol (4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: the spots obtained from the sample solution and the standard solution show a red-purple color and the same *R*f value, and any spot from the sample solution other than that corresponding to the spot from the standard solution does not appear.

Loss on drying <*2.41*> Not more than 2.0% (0.2 g, silica gel, 4 hours).

Assay Weigh accurately about 10 mg each of Ergometrine Maleate and Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water to make exactly 250 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 2 mL of each solution into a separate brown glass-stoppered tube. To each tube add 4 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, exactly measured, while cooling in an ice bath, then warm at 45°C for 10 minutes. Allow to stand at room temperature for 20 minutes, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 2 mL of water in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 550 nm, respectively.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Ergometrine Maleate RS taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ergometrine Maleate Injection

エルゴメトリンマレイン酸塩注射液

Ergometrine Maleate Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$: 441.48).

Method of preparation Prepare as directed under Injections, with Ergometrine Maleate.

Description Ergometrine Maleate Injection is a clear, colorless to pale yellow liquid.

pH: 2.7 – 3.5

Identification **(1)** Measure a volume of Ergometrine Maleate Injection, equivalent to 3 mg of Ergometrine Maleate, if necessary, dilute with water or evaporate on a water bath to make 15 mL, and use this solution as the sample solution. The sample solution shows a blue fluorescence.

(2) To 1 mL of the sample solution obtained in (1) add 1 mL of ammonia TS, and extract with 20 mL of diethyl ether. To the diethyl ether extract add 1 mL of dilute sulfuric acid, shake, and warm to remove diethyl ether in a water bath. Cool, to the residue obtained add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and allow to stand for 5 to 10 minutes: a deep blue color develops.

(3) To 5 mL of the sample solution obtained in (1) add 1 drop of potassium permanganate TS: a red color disappears immediately.

Bacterial endotoxins <*4.01*> Less than 1500 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer an exactly measured volume of Ergometrine Maleate Injection, equivalent to about 2 mg of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$), and add sodium chloride in a ratio of 0.3 g to 1 mL of the solution. To this mixture add 20 mL of diethyl ether and 2 mL of ammonia TS, shake, and extract. Further, extract with three 15-mL portions of diethyl ether, combine all the extracts, add 5 g of anhydrous sodium sulfate, filter through a pledget of absorbent cotton, and wash with three 5-mL portions of diethyl ether. Add the washings to the filtrate, shake with 5 mL of dilute sulfuric acid, evaporate the diethyl ether by warming in a current of nitrogen, to the remaining solution add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 2 mg of Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, add water to make exactly 50 mL, and use this solution as the standard solution. Transfer 2 mL each of the sample solution and standard solution, accurately measured, to separate glass-stoppered test tubes, and proceed as directed in the Assay under Ergometrine Maleate.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Ergometrine Maleate RS taken

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, and in a cold place.

Ergometrine Maleate Tablets

エルゴメトリンマレイン酸塩錠

Ergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$: 441.48).

Method of preparation Prepare as directed under Tablets, with Ergometrine Maleate.

Identification To a quantity of powdered Ergometrine Maleate Tablets, equivalent to 3 mg of Ergometrine Maleate, add 15 mL of warm water, shake, and filter: the filtrate shows a blue fluorescence. Proceed with this solution as directed in the Identification (2) and (3) under Ergometrine Maleate.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Ergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, and add exactly V mL of a solution of L-tartaric acid (1 in 100) so that each mL contains about 40 μg of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$). Stopper the tube, shake for 30 minutes vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 4 mg of Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution into separate brown glass-stoppered test tubes, add exactly 8 mL each of 4-dimethylaminobenzaldehyde-iron (III) chloride TS while cooling in an ice bath, after shaking, and allow to stand for 1 hour at ordinary temperature. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with 4 mL of water in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 550 nm, respectively.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$) $= M_S \times A_T/A_S \times V/100$

M_S: Amount (mg) of Ergometrine Maleate RS taken

Assay Weigh accurately, and powder not less the 20 Ergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 2 mg of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$), transfer to a glass filter (G4), add 10 mL of a solution of L-tartaric acid (1 in 100), and filter with thorough shaking. Repeat the procedures 3 times, combine the filtrates, add a solution of L-tartaric acid (1 in 100) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 2 mg of Ergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in a solution of L-tartaric acid (1 in 100) to make exactly 50 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution, and proceed as directed in the Assay under Ergometrine Maleate.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_3O_2.C_4H_4O_4$) $= M_S \times A_T/A_S$

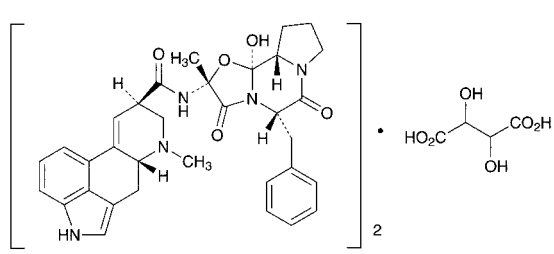
M_S: Amount (mg) of Ergometrine Maleate RS taken

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Ergotamine Tartrate

エルゴタミン酒石酸塩



$(C_{33}H_{35}N_5O_5)_2.C_4H_6O_6$: 1313.41

(5′*S*)-5′-Benzyl-12′-hydroxy-2′-methylergotaman-3′,6′,18-trione hemitartrate

[*379-79-3*]

Ergotamine Tartrate contains not less than 98.0% of ergotamine tartrate [$(C_{33}H_{35}N_5O_5)_2.C_4H_6O_6$], calculated on the dried basis.

Description Ergotamine Tartrate occurs as colorless crystals, or a white to pale yellowish white or grayish white crystalline powder.

It is slightly soluble in water and in ethanol (95).

Melting point: about 180°C (with decomposition).

Identification **(1)** Dissolve 1 mg of Ergotamine Tartrate in 10 mL of a mixture of acetic acid (100) and ethyl acetate (1:1). To 0.5 mL of this solution add slowly 0.5 mL of sulfuric acid, with shaking in cold water, and allow to stand: a

purple color develops. To this solution add 0.1 mL of diluted iron (III) chloride TS (1 in 12): the color of the solution changes to blue to blue-purple.

(2) Dissolve 1 mg of Ergotamine Tartrate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake: a blue color develops.

Optical rotation ⟨*2.49*⟩ Ergotamine base $[\alpha]_D^{20}$: −155 – −165°. Dissolve 0.35 g of Ergotamine Tartrate in 25 mL of a solution of L-tartaric acid (1 in 100), add 0.5 g of sodium hydrogen carbonate, shake gently and sufficiently, and extract with four 10-mL portions of ethanol-free chloroform. Filter the extracts successively through a small filter paper, moistened with ethanol-free chloroform, into a 50-mL volumetric flask. Allow the flask to stand in a water bath at 20°C for 10 minutes, and determine the optical rotation in a 100-mm cell. Separately, pipet 25 mL of this solution, evaporate to dryness under reduced pressure at a temperature not higher than 45°C, dissolve the residue in 25 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination, and make any necessary correction. Calculate the specific rotation of the ergotamine base from the consumed volume of 0.05 mol/L perchloric acid VS and the optical rotation.

Each mL of 0.05 mol/L perchloric acid VS
= 29.08 mg of $C_{33}H_{35}N_5O_5$

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. To 40 mg of Ergotamine Tartrate add 10 mL of a solution of L-tartaric acid in diluted methanol (1 in 2) (1 in 1000), dissolve with thorough shaking, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-tartaric acid in diluted methanol (1 in 2) (1 in 1000) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.1 g, in vacuum, 60°C, 4 hours).

Assay Weigh accurately about 0.2 g of Ergotamine Tartrate, dissolve in 15 mL of a mixture of acetic acid (100) and acetic anhydride (50:3), and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination, and make any necessary correction.

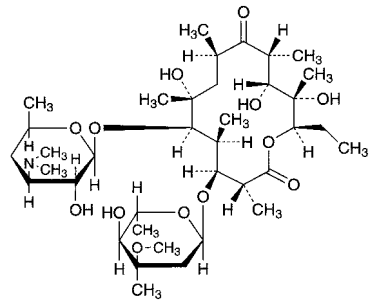
Each mL of 0.05 mol/L perchloric acid VS
= 32.84 mg of $(C_{33}H_{35}N_5O_5)_2.C_4H_6O_6$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere, and not exceeding 5°C.

Erythromycin

エリスロマイシン



$C_{37}H_{67}NO_{13}$: 733.93
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide
[*114-07-8*]

Erythromycin is a macrolide substance having antibacterial activity produced by the growth of *Saccharopolyspora erythraea*.

It contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$).

Description Erythromycin occurs as a white to light yellowish white powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in methanol and in ethanol (95), and very slightly soluble in water.

Identification **(1)** Determine the infrared absorption spectrum of Erythromycin as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Erythromycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg each of Erythromycin and Erythromycin RS in 1 mL of methanol, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (50:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 100°C for 15 minutes: the principal spot from the sample solution and the spot from the standard solution are dark purple in color, and their *R*f values are the same.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −71 – −78° (1 g calculated on the anhydrous basis, ethanol (95), 50 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Erythromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Erythromycin according to Method 5 using hydrochloric

acid instead of diluted hydrochloric acid (1 in 2), and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 40 mg of Erythromycin in 2 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 16 mg of Erythromycin RS in 2 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 10 mL, and use this solution as the standard stock solution. Dissolve 5 mg each of erythromycin B and erythromycin C in 2 mL of methanol, add exactly 2 mL of the standard stock solution, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of erythromycin B and erythromycin C from the sample solution are not larger than those of erythromycin B and erythromycin C from the standard solution, respectively, and each area of the peaks other than erythromycin, erythromycin B and erythromycin C is not larger than the area of the peak of erythromycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 70°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 9.0 with diluted phosphoric acid (1 in 10). To 50 mL of this solution add 190 mL of *t*-butylalcohol, 30 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust so that the retention time of erythromycin is about 20 minutes.

Time span of measurement: About 4 times as long as the retention time of erythromycin, beginning after the solvent peak.

System suitability—

System performance: Dissolve 2 mg of *N*-demethylerythromycin in 10 mL of the standard solution. When the procedure is run with 100 μL of this solution under the above operating conditions, *N*-demethylerythromycin, erythromycin C, erythromycin and erythromycin B are eluted in this order, with the resolution between the peaks of *N*-demethylerythromycin and erythromycin C being not less than 0.8, and with the resolution between the peaks of *N*-demethylerythromycin and erythromycin being not less than 5.5.

System repeatability: When the test is repeated 3 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of erythromycin is not more than 3.0%.

Water <*2.48*> Not more than 10.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Erythromycin RS, equivalent to about 25 mg (potency), dissolve in 25 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin, equivalent to about 25 mg (potency), dissolve in 25 mL of methanol, and add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Erythromycin Delayed-release Tablets

エリスロマイシン腸溶錠

Erythromycin Delayed-release Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Method of preparation Prepare as directed under Tablets, with Erythromycin.

Identification To a quantity of powdered Erythromycin Delayed-release Tablets, equivalent to 10 mg (potency) of Erythromycin, add 1 mL of methanol, shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of Erythromycin RS in 1 mL of methanol, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Erythromycin.

Loss on drying <*2.41*> Not more than 10.0% (0.2 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Disintegration <*6.09*> It meets the requirement. For the test with 2nd fluid for disintegration test, use the disk.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Erythromycin.

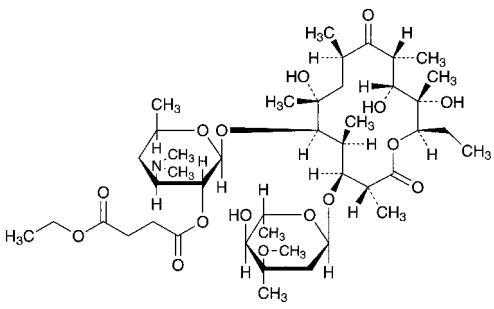
(ii) Sample solutions—Weigh accurately the mass of not less than 20 Erythromycin Delayed-release Tablets, and

powder. Weigh accurately a portion of the powder, equivalent to about 25 mg (potency) of Erythromycin, add 25 mL of methanol, shake vigorously, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL, and filter. Take exactly an appropriate volume of the filtrate, add 0.1 mol/L phosphate buffer solution (pH 8.0) to prepare solutions containing 20 μg (potency) and 5 μg (potency) per mL, and use these solutions as the high and the low concentration sample solutions, respectively.

Containers and storage Containers—Well-closed containers.

Erythromycin Ethylsuccinate

エリスロマイシンエチルコハク酸エステル



$C_{43}H_{75}NO_{16}$: 862.05
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-[3,4,6-Trideoxy-2-*O*-(3-ethoxycarbonylpropanoyl)-3-dimethylamino-β-D-*xylo*-hexopyranosyloxy]-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide
[*41342-53-4*]

Erythromycin Ethylsuccinate is a derivative of erythromycin.

It contains not less than 780 μg (potency) and not more than 900 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Ethylsuccinate is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Description Erythromycin Ethylsuccinate occurs as a white powder.

It is freely soluble in methanol and in acetone, soluble in ethanol (95), and practically insoluble in water.

Identification (1) Dissolve 3 mg of Erythromycin Ethylsuccinate in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and is immediately changed to red to deep purple.

(2) Determine the infrared absorption spectrum of Erythromycin Ethylsuccinate, previously dried in a desiccator (reduced pressure, silica gel) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water ⟨*2.48*⟩ Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

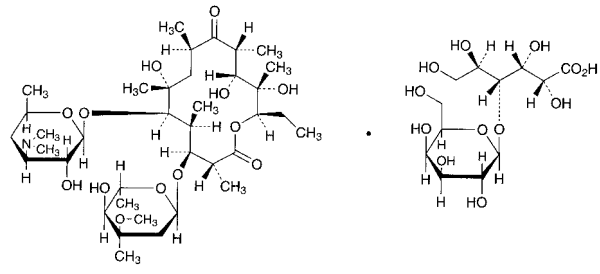
(iii) Standard solutions—Weigh accurately an amount of Erythromycin RS, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Ethylsuccinate, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Erythromycin Lactobionate

エリスロマイシンラクトビオン酸塩



$C_{37}H_{67}NO_{13}.C_{12}H_{22}O_{12}$: 1092.22
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide mono(4-*O*-β-D-galactopyranosyl-D-gluconate)
[*3847-29-8*]

Erythromycin Lactobionate is the lactobionate of erythromycin.

It contains not less than 590 μg (potency) and not more than 700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Lactobionate is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Description Erythromycin Lactobionate occurs as a white powder.

It is freely soluble in water, in methanol and in ethanol (99.5), and very slightly soluble in acetone.

Identification (1) To 3 mg of Erythromycin Lactobionate add 2 mL of acetone, and add 2 mL of hydrochloric acid: an

orange color is produced, and it changes immediately to red to deep purple.

(2) Transfer about 0.3 g of Erythromycin Lactobionate to a separator, add 15 mL of ammonia TS and 15 mL of chloroform, shake, and take the separated aqueous layer. Wash the aqueous layer with three 15-mL portions of chloroform, and evaporate the aqueous liquid on a water bath to dryness. Dissolve the residue in 10 mL of a mixture of methanol and water (3:2), and use this solution as the sample solution. Separately, dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer obtained from a mixture of water, 1-butanol and acetic acid (100) (3:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid, and heat at 105°C for 20 minutes: the principal spot obtained from the sample solution shows a deep brown and the *R*f value which are the same as those of the principal spot obtained from the standard solution.

pH <*2.54*> The pH of a solution obtained by dissolving 0.5 g of Erythromycin Lactobionate in 10 mL of water is between 5.0 and 7.5.

Water <*2.48*> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

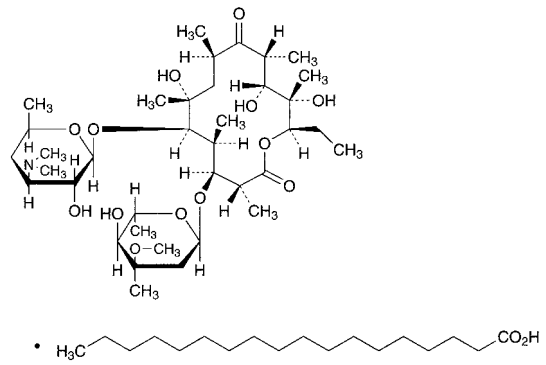
(iii) Standard solutions—Weigh accurately an amount of Erythromycin RS, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Lactobionate, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Erythromycin Stearate

エリスロマイシンステアリン酸塩



$C_{37}H_{67}NO_{13}.C_{18}H_{36}O_2$: 1018.40

(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide monostearate

[*643-22-1*]

Erythromycin Stearate is the stearate of erythromycin.

It contains not less than 600 μg (potency) and not more than 720 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Stearate is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Description Erythromycin Stearate occurs as a white powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, and practically insoluble in water.

Identification **(1)** Dissolve 3 mg of Erythromycin Stearate in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and is immediately changed to red to deep purple.

(2) Determine the infrared absorption spectrum of Erythromycin Stearate, previously dried in a desiccator (reduced pressure, silica gel) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water <*2.48*> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Erythromycin RS equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days.

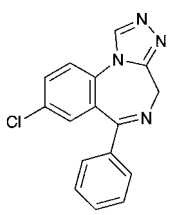
Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Stearate equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Estazolam

エスタゾラム



$C_{16}H_{11}ClN_4$: 294.74
8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
[*29975-16-4*]

Estazolam, when dried, contains not less than 98.5% of estazolam ($C_{16}H_{11}ClN_4$).

Description Estazolam occurs as white to pale yellowish white, crystals or crystalline powder. It is odorless, and has a bitter taste.

It is soluble in methanol and in acetic anhydride, sparingly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification **(1)** Dissolve 0.01 g of Estazolam in 3 mL of sulfuric acid: the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Estazolam in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Estazolam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 229 – 233°C

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Estazolam in 10 mL of ethanol (95): the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 1.0 g of Estazolam in 10 mL of ethanol (95) by heating, add 40 mL of water, cool with shaking in ice water, allow to stand to attain ordinary temperature, and filter. To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of ethanol (95) (not more than 0.015%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Estazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Estazolam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Estazolam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform and methanol (5:3:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the principal spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (2 g).

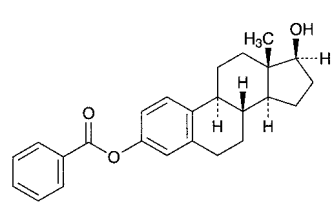
Assay Weigh accurately about 0.25 g of Estazolam, previously dried, dissolve in 100 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration), until the solution changes to the second equivalence point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.74 mg of $C_{16}H_{11}ClN_4$

Containers and storage Containers—Well-closed containers.

Estradiol Benzoate

エストラジオール安息香酸エステル



$C_{25}H_{28}O_3$: 376.49
Estra-1,3,5(10)-triene-3,17β-diol 3-benzoate
[*50-50-0*]

Estradiol Benzoate, when dried, contains not less than 97.0% of estradiol benzoate ($C_{25}H_{28}O_3$).

Description Estradiol Benzoate occurs as a white crystalline powder. It is odorless.

It is sparingly soluble in acetone, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification **(1)** To 2 mg of Estradiol Benzoate add 2 mL of sulfuric acid: a yellowish green color with a blue fluorescence is produced, and the color of the solution changes to light orange on the careful addition of 2 mL of

water.

(2) Determine the infrared absorption spectrum of Estradiol Benzoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Estradiol Benzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +54 – +58° (after drying, 0.1 g, acetone, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 191 – 198°C

Purity **(1)** 3,17α-Estradiol—Dissolve 5.0 mg each of Estradiol Benzoate and Estradiol Benzoate RS in acetone to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Place exactly 2 mL each of the sample solution and standard solution in separate glass-stoppered test tube, add boiling stones, evaporate the acetone by heating in a water bath, and dry the residue in a desiccator (in vacuum, phosphorus (V) oxide) for 1 hour. Add 1.0 mL of dilute iron-phenol TS to each test tube. Stopper the test tubes loosely, heat for 30 seconds in a water bath, shake in a water bath for several seconds, and heat for 2 minutes. Cool the solutions in ice for 2 minutes, add 4.0 mL of diluted sulfuric acid (7 in 20), and mix well: the solution obtained from the sample solution has no more color than that obtained from the standard solution.

(2) Related substances—Dissolve 40 mg of Estradiol Benzoate in 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.1 g).

Assay Weigh accurately about 10 mg each of Estradiol Benzoate and Estradiol Benzoate RS, previously dried, and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, add 5 mL of the internal standard solution, then add methanol to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of estradiol benzoate to that of the internal standard.

$$\text{Amount (mg) of estradiol benzoate } (C_{25}H_{28}O_3) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Estradiol Benzoate RS taken

Internal standard solution—A solution of progesterone in methanol (13 in 80,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust so that the retention time of estradiol benzoate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and estradiol benzoate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estradiol benzoate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Estradiol Benzoate Injection (Aqueous Suspension)

エストラジオール安息香酸エステル水性懸濁注射液

Estradiol Benzoate Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of estradiol benzoate ($C_{25}H_{28}O_3$: 376.49).

Method of preparation Prepare as directed under Injection, with Estradiol Benzoate.

Description Estradiol Benzoate Injection (Aqueous Suspension) produces a white turbidity on shaking.

Identification Extract a volume of Estradiol Benzoate Injection (Aqueous Suspension), equivalent to 1 mg of Estradiol Benzoate, with 5 mL of chloroform, and use this extract as the sample solution. Separately, dissolve 1 mg of Estradiol Benzoate RS in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Direct inoculation method: it meets the requirement.

Assay Measure exactly a volume of well-mixed Estradiol Benzoate Injection (Aqueous Suspension), equivalent to about 2 mg of estradiol benzoate ($C_{25}H_{28}O_3$), dissolve the crystals with an appropriate quantity of methanol, and add methanol to make exactly 20 mL. Pipet 10 mL of this solu-

tion, add exactly 10 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Estradiol Benzoate RS, previously dried in desiccator (reduced pressure, phosphorus (V) oxide) for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the standard solution. Proceed with these solutions as directed in the Assay under Estradiol Benzoate.

$$\text{Amount (mg) of estradiol benzoate } (C_{25}H_{28}O_3) = M_S \times Q_T/Q_S \times 1/5$$

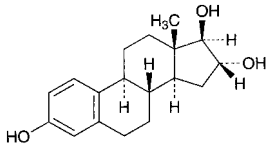
M_S: Amount (mg) of Estradiol Benzoate RS taken

Internal standard solution—A solution of progesterone in methanol (13 in 100,000).

Containers and storage Containers—Hermetic containers.

Estriol

エストリオール



$C_{18}H_{24}O_3$: 288.38
Estra-1,3,5(10)-triene-3,16α,17β-triol
[*50-27-1*]

Estriol, when dried, contains not less than 97.0% and not more than 102.0% of estriol ($C_{18}H_{24}O_3$).

Description Estriol occurs as a white crystalline powder. It is odorless.

It is sparingly soluble in methanol, slightly soluble in ethanol (95) and in 1,4-dioxane, and practically insoluble in water and in diethyl ether.

Identification (1) Dissolve 0.01 g of Estriol in 100 mL of ethanol (95) by warming, and use this solution as the sample solution. Evaporate 1 mL of the sample solution on a water bath to dryness, add 5 mL of a solution of sodium *p*-phenolsulfonate in diluted phosphoric acid (1 in 50), heat at 150°C for 10 minutes, and cool: a red-purple color develops.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Estriol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Estriol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Estriol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +54 – +62° (after drying, 40 mg, 1,4-dioxane, 10 mL, 100 mm).

Melting point <*2.60*> 281 – 286°C

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of Estriol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 40 mg of Estriol in 10 mL of ethanol (95) by warming, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and acetic acid (100) (18:1:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 25 mg each of Estriol and Estriol RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of estriol to that of the internal standard.

$$\text{Amount (mg) of estriol } (C_{18}H_{24}O_3) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Estriol RS taken

Internal standard solution—A solution of methyl benzoate for estriol test in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (51:49).

Flow rate: Adjust so that the retention time of estriol is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, estriol and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estriol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Estriol Injection (Aqueous Suspension)

エストリオール水性懸濁注射液

Estriol Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of estriol ($C_{18}H_{24}O_3$: 288.38).

Method of preparation Prepare as directed under Injections, with Estriol.

Description Shake Estriol Injection (Aqueous Suspension): a white turbidity is produced.

Identification (1) Shake well, take a volume of Estriol Injection (Aqueous Suspension), equivalent to 2 mg of Estriol, add ethanol (95) to make 20 mL, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obrtained in (1) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 279 nm and 283 nm.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Sterility <*4.06*> Perform the test according to the Direct inoculation method: it meets the requirement.

Assay Shake well, pipet a volume of Estriol Injection (Aqueous Suspension), equivalent to about 5 mg of estriol ($C_{18}H_{24}O_3$), and dissolve in methanol to make exactly 20 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Estriol RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Estriol.

$$\text{Amount (mg) of estriol } (C_{18}H_{24}O_3) = M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of Estriol RS taken

Internal standard solution—A solution of methyl benzoate for estriol test in ethanol (95) (1 in 5000).

Containers and storage Containers—Hermetic containers.

Estriol Tablets

エストリオール錠

Estriol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of estriol ($C_{18}H_{24}O_3$: 288.38).

Method of preparation Prepare as directed under Tablets, with Estriol.

Identification (1) Weigh a portion of powdered Estriol Tablets, equivalent to 2 mg of Estriol, add 20 mL of ethanol (95), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 279 nm and 283 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Estriol Tablets add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, add exactly 15 mL of methanol, and shake for 15 minutes. Centrifuge this solution for 10 minutes, pipet a definite amount of the supernatant liquid, and add methanol to make exactly a definite amount of solution so that each ml of the solution contains about 5 μg of estriol ($C_{18}H_{24}O_3$). Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Proceed with 20 μL of the sample solution as directed in the Assay under Estriol. Use a solution of methyl benzoate for estriol test in methanol (1 in 40,000) as the internal standard solution. Calculate the mean value from each ratio of peak areas of 10 samples: the samples conform to the requirements if the deviation (%) of the mean value and each ratio of peak areas is within 15%. If the deviation (%) exceeds 15%, and 1 sample shows deviation within 25%, repeat the test with 20 samples. Calculate the deviation (%) of the mean value from each ratio of peak areas of the 30 samples used in the 2 tests and each ratio of peak areas: the samples conform to the requirements if the deviation exceeds 15%, not more than 1 sample shows deviation within 25%, and no sample shows deviation exceeding 25%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Estriol Tablets is not less than 80%.

Start the test with 1 tablet of Estriol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.1 μg of estriol ($C_{18}H_{24}O_3$), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Estriol RS, previously dried at 105°C for 3 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of estriol.

$$\text{Dissolution rate (\%) with respect to the labeled amount of estriol } (C_{18}H_{24}O_3) = M_S \times A_T/A_S \times V'/V \times 1/C \times 9/10$$

M_S: Amount (mg) of Estriol RS taken

C: Labeled amount (mg) of estriol ($C_{18}H_{24}O_3$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Estriol.

System suitability—

Proceed as directed in the system suitability in the Assay under Estriol.

Assay Weigh accurately and powder not less than 20 Estriol Tablets. Weigh accurately a portion of the powder, equivalent to about 1 mg of estriol ($C_{18}H_{24}O_3$), add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, shake with 25 mL of methanol for 10 minutes, centrifuge, and take the supernatant liquid. Add 25 mL of methanol, repeat the above procedure twice, combine the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Estriol RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the standard solution. Proceed with 20 μL each of the sample solution and standard solution as directed in the Assay under Estriol.

$$\text{Amount (mg) of estriol } (C_{18}H_{24}O_3) = M_S \times Q_T/Q_S \times 1/25$$

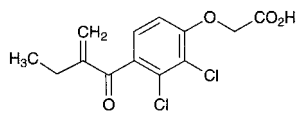
M_S: Amount (mg) of Estriol RS taken

Internal standard solution—A solution of methyl benzoate for estriol test in methanol (1 in 5000).

Containers and storage Containers—Tight containers.

Etacrynic Acid

エタクリン酸



$C_{13}H_{12}Cl_2O_4$: 303.14

[2,3-Dichloro-4-(2-ethylacryloyl)phenoxy]acetic acid

[*58-54-8*]

Etacrynic Acid, when dried, contains not less than 98.0% of etacrynic acid ($C_{13}H_{12}Cl_2O_4$).

Description Etacrynic Acid occurs as a white crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95), in acetic acid (100) and in diethyl ether, and very slightly soluble in water.

Identification **(1)** Dissolve 0.2 g of Etacrynic Acid in 10 mL of acetic acid (100), and to 5 mL of this solution add 0.1 mL of bromine TS: the color of the test solution disappears. To the remaining 5 mL of the solution add 0.1 mL of potassium permanganate TS: the color of the test solution changes to light orange immediately.

(2) To 0.01 g of Etacrynic Acid add 1 mL of sodium hydroxide TS, and heat in a water bath for 3 minutes. After cooling, add 1 mL of chromotropic acid TS, and heat in a water bath for 10 minutes: a deep purple color develops.

(3) Determine the absorption spectrum of a solution of Etacrynic Acid in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Etacrynic Acid as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 121 – 125°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Etacrynic Acid in 10 mL of methanol: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Etacrynic Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Etacrynic Acid according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Etacrynic Acid in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (6:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.25% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Etacrynic Acid, previously dried, place in an iodine bottle, dissolve in 20 mL of acetic acid (100), and add exactly 20 mL of 0.05 mol/L bromine VS. To this solution add 3 mL of hydrochloric acid, stopper tightly at once, shake, and allow to stand in a dark place for 60 minutes. Add carefully 50 mL of water and 15 mL of potassium iodide TS, stopper tightly at once, shake well, and titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Each mL of 0.05 mol/L bromine VS} = 15.16 \text{ mg of } C_{13}H_{12}Cl_2O_4$$

Containers and storage Containers—Well-closed containers.

Etacrynic Acid Tablets

エタクリン酸錠

Etacrynic Acid Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of etacrynic acid ($C_{13}H_{12}Cl_2O_4$: 303.14).

Method of preparation Prepare as directed under Tablets, with Etacrynic Acid.

Identification **(1)** Weigh a quantity of powdered Etacrynic Acid Tablets, equivalent to 0.3 g of Etacrynic Acid, add 25 mL of 0.1 mol/L hydrochloric acid TS, and extract with 50 mL of dichloromethane. Filter the dichloromethane

extract, and evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1), (2) and (4) under Etacrynic Acid.

(2) Prepare a solution of the residue obtained in (1), equivalent to a solution of Etacrynic Acid in methanol (1 in 20,000), and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 268 nm and 272 nm.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Etacrynic Acid Tablets is not less than 70%.

Start the test with 1 tablet of Etacrynic Acid Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 28 μg of etacrynic acid ($C_{13}H_{12}Cl_2O_4$), and use this solution as the sample solution. Separately, weigh accurately about 55 mg of etacrynic acid for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 277 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of etacrynic acid ($C_{13}H_{12}Cl_2O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of etacrynic acid for assay taken
C: Labeled amount (mg) of etacrynic acid ($C_{13}H_{12}Cl_2O_4$) in 1 tablet

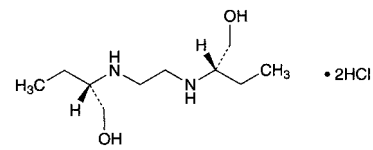
Assay Weigh accurately and powder not less than 20 Etacrynic Acid Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of etacrynic acid ($C_{13}H_{12}Cl_2O_4$), add 25 mL of 0.1 mol/L hydrochloric acid TS, and extract with three 30-mL portions of dichloromethane. Filter the dichloromethane extracts through a pledget of absorbent cotton into an iodine bottle. Wash the pledget of absorbent cotton with a small amount of dichloromethane, and combine the washing with the extracts. Evaporate this solution on a water bath to dryness in a current of air, to the residue add 20 mL of acetic acid (100), and proceed as directed in the Assay under Etacrynic Acid.

Each mL of 0.05 mol/L bromine VS
= 15.16 mg of $C_{13}H_{12}Cl_2O_4$

Containers and storage Containers—Well-closed containers.

Ethambutol Hydrochloride

エタンブトール塩酸塩



$C_{10}H_{24}N_2O_2.2HCl$: 277.23
(2*S*,2′*S*)-2,2′-(Ethylenediimino)bis(butan-1-ol) dihydrochloride
[*1070-11-7*]

Ethambutol Hydrochloride, when dried, contains not less than 98.5% of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2.2HCl$).

Description Ethambutol Hydrochloride occurs as white, crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution prepared by dissolving 1.0 g of Ethambutol Hydrochloride in 20 mL of water is between 3.4 and 4.0.

Identification **(1)** To 10 mL of a solution of Ethambutol Hydrochloride (1 in 100) add 0.5 mL of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a deep blue color is produced.

(2) Dissolve 0.1 g of Ethambutol Hydrochloride in 40 mL of water, add 20 mL of 2,4,6-trinitrophenol TS, and allow to stand for 1 hour. Collect the precipitate, wash with 50 mL of water, and dry at 105°C for 2 hours: the precipitate melts ⟨*2.60*⟩ between 193°C and 197°C.

(3) A solution of Ethambutol Hydrochloride (1 in 30) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +5.5 – +6.1° (after drying, 5 g, water, 50 mL, 200 mm).

Melting point ⟨*2.60*⟩ 200 – 204°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ethambutol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g Ethambutol Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Ethambutol Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) 2-Aminobutanol—Dissolve 5.0 g of Ethambutol Hydrochloride in methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of 2-amino-1-butanol in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100), hydrochloric acid and water (11:7:1:1) to a distance of about 10 cm, air-dry the plate, and heat at 105°C for 5 minutes. Cool, spray evenly ninhydrin-L-ascorbic acid TS upon the plate, air-dry the plate, and heat at 105°C for 5 minutes: the spot from the sample solution,

corresponding to that from the standard solution, has no more color than that from the standard solution.

Loss on drying ⟨2.41⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ethambutol Hydrochloride, previously dried, dissolve in 20 mL of water, and add 1.8 mL of copper (II) sulfate TS. To the solution add 7 mL of sodium hydroxide TS with shaking, add water to make exactly 50 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add 10 mL of ammonia-ammonium chloride buffer solution (pH 10.0) and 100 mL of water, and titrate ⟨2.50⟩ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from blue-purple through light red to light yellow (indicator: 0.15 mL of Cu-PAN TS). Perform a blank determination, and make any necessary correction.

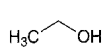
Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.772 mg of $C_{10}H_{24}N_2O_2.2HCl$

Containers and storage Containers—Tight containers.

Ethanol

Alcohol

エタノール



C_2H_6O: 46.07
Ethanol
[*64-17-5*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Ethanol contains not less than 95.1 vol% and not more than 96.9 vol% (by specific gravity) of ethanol (C_2H_6O) at 15°C.

◆**Description** Ethanol is a clear, colorless liquid.

It is miscible with water.

It is flammable and burns with a light blue flame on ignition.

It is volatile.◆

Identification Determine the infrared absorption spectrum of Ethanol as directed in the liquid film method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity ⟨2.56⟩ d_{15}^{15}: 0.80872 – 0.81601

Purity **(1)** Clarity and color of solution—Ethanol is clear and colorless. To 1.0 mL of Ethanol add water to make 20 mL, and allow to stand for 5 minutes: the resulting liquid is clear. Control solution: water.

(2) Acidity or alkalinity—To 20 mL of Ethanol add 20 mL of freshly boiled and cooled water and 0.1 mL of a solution prepared by addition of 7.0 mL of ethanol (95) and 2.0 mL of water to 1.0 mL of phenolphthalein TS : no color develops. Add 1.0 mL of 0.01 mol/L sodium hydroxide VS to this solution: a pink color develops.

(3) Volatile impurities—Pipet 500 mL of Ethanol, add 150 μL of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 μL of anhydrous methanol add Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to exactly 50 μL each of anhydrous methanol and acetaldehyde add Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to exactly 150 μL of acetal add Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to exactly 100 μL of benzene add Ethanol to make exactly 100 mL. To exactly 100 μL of this solution add Ethanol to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with exactly 1 μL each of Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography ⟨2.02⟩ according to the following conditions, and determine the peak areas of acetaldehyde, A_E, benzene, B_E and acetal, C_E obtained with Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, A_T with the standard solution (2), the peak area of acetal, C_T with the standard solution (3) and the peak area of benzene, B_T with the standard solution (4): the peak area of methanol obtained with Ethanol is not larger than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above is not larger than the peak area of 4-methylpentan-2-ol. For this calculation the peak having the area not more than 3% that of 4-methylpentan-2-ol is excluded.

Total amount (vol ppm) of acetaldehyde and acetal
$= (10 \times A_E)/(A_T - A_E) + (30 \times C_E \times 44.05)/\{(C_T - C_E) \times 118.2\}$

Amount (vol ppm) of benzene $= 2B_E/(B_T - B_E)$

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94%dimethyl silicone polymer for gas chromatography in 1.8 μm thickness.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 12 minutes, then rise up to 240°C at the rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Carrier gas: Helium.

Flow rate: 35 cm per second.

Split ratio: 1: 20.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

(4) Other impurities (absorbance)—Determine the absorption spectrum of Ethanol between 235 nm and 340 nm as

directed under Ultraviolet-visible Spectrophotometry <2.24>, in a 5-cm cell using water as a blank: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively, and the spectrum shows a steadily descending curve with no observable peaks or shoulders.

(5) Residue on evaporation—Evaporate 100 mL of Ethanol, exactly measured, in a tared dish on a water bath, and dry at 105°C for 1 hour: the mass of the residue does not exceed 2.5 mg.

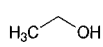
Containers and storage ◆Containers—Tight containers.◆ Storage—Without exposure to light.

◆**Shelf life** In not glass containers: Unless otherwise specified, 24 months after preparation.◆

Anhydrous Ethanol

Dehydrated Alcohol

無水エタノール



C_2H_6O: 46.07
Ethanol
[*64-17-5*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Anhydrous Ethanol contains not less than 99.5 vol% (by specific gravity) of ethanol (C_2H_6O) at 15°C.

◆**Description** Anhydrous Ethanol is a clear, colorless liquid.

It is miscible with water.

It is flammable and burns with a light blue flame on ignition.

It is volatile.

Boiling point: 78 – 79°C◆

Identification Determine the infrared absorption spectrum of Anhydrous Ethanol as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d_{15}^{15}: 0.79422 – 0.79679

Purity **(1)** Clarity and color of solution—Anhydrouse Ethanol is clear and colorless. To 1.0 mL of Anhydrous Ethanol add water to make 20 mL, and allow to stand for 5 minutes: the resulting liquid is clear. Control solution: water

(2) Acidity or alkalinity—To 20 mL of Anhydrous Ethanol add 20 mL of freshly boiled and cooled water and 0.1 mL of a solution obtained by addition of 7.0 mL of ethanol (95) and 2.0 mL of water to 1.0 mL of phenolphthalein TS: no color develops. Add 1.0 mL of 0.01 mol/L sodium hydroxide VS to this solution: pink color develops.

(3) Volatile impurities—Pipet 500 mL of Anhydrous Ethanol, add 150 μL of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 μL of anhydrous methanol add Anhydrous Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Anhydrous Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to exactly 50 μL each of anhydrous methanol and acetaldehyde add Anhydrous Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to exactly 150 μL of acetal add Anhydrous Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to exactly 100 μL of benzene add Anhydrous Ethanol to make exactly 100 mL. To exactly 100 μL of this solution add Anhydrous Ethanol to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with exactly 1 μL each of Anhydrous Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of acetaldehyde, A_E, benzene, B_E and acetal, C_E obtained with Anhydrous Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, A_T with the standard solution (2), the peak area of acetal, C_T with the standard solution (3) and the peak area of benzene, B_T with the standard solution (4): the peak area of methanol obtained with Anhydrous Ethanol is not larger than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above is not larger than the peak area of 4-methylpentan-2-ol. For this calculation the peak having the area not more than 3% that of 4-methylpentan-2-ol is excluded.

$$\text{Total amount (vol ppm) of acetaldehyde and acetal} = (10 \times A_E)/(A_T - A_E) + (30 \times C_E \times 44.05)/\{(C_T - C_E) \times 118.2\}$$

$$\text{Amount (vol ppm) of benzene} = 2B_E/(B_T - B_E)$$

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94%dimethyl silicone polymer for gas chromatography in 1.8 μm thickness.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 12 minutes, then rise up to 240°C at the rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Carrier gas: Helium.

Flow rate: 35 cm per second.

Split ratio: 1: 20.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

(4) Other impurities (absorbance)—Determine the absorption spectrum of Anhydrous Ethanol between 235 nm and 340 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, in a 5-cm cell using water as a blank: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively, and the spectrum shows a steadily

descending curve with no observable peaks or shoulders.

(5) Residue on evaporation—Evaporate 100 mL of Anhydrous Ethanol, exactly measured, in a tared dish on a water bath, and dry at 105°C for 1 hour: the mass of the residue does not exceed 2.5 mg.

Containers and storage ◆Containers—Tight containers.◆
Storage—Without exposure to light.

◆**Shelf life** In not glass containers: Unless otherwise specified, 24 months after preparation.◆

Ethanol for Disinfection

Alcohol for Disinfection

消毒用エタノール

Ethanol for Disinfection contains not less than 76.9 vol% and not more than 81.4 vol% (by specific gravity) of ethanol (C_2H_6O: 46.07) at 15°C.

Method of preparation

Ethanol	830 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare by mixing the above ingredients.

Description Ethanol for Disinfection is a colorless, clear liquid.
It is miscible with water.
It burns with a light blue flame on ignition.
It is volatile.

Identification (1) To 1 mL of Ethanol for Disinfection add 2 mL of iodine TS and 1 mL of sodium hydroxide TS, and mix: light yellow precipitates appear.

(2) To 1 mL of Ethanol for Disinfection add 1 mL of acetic acid (100) and 3 drops of sulfuric acid, and heat: the odor of ethyl acetate is produced.

Specific gravity ⟨*2.56*⟩ d_{15}^{15}: 0.86027 – 0.87264

Purity Proceed as directed in the Purity under Ethanol, with the exeption of (4), which is changed as follows.

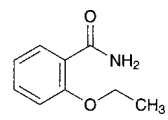
(4) Other impurities (absorbance)—Perform the test with Ethanol for Disinfection as directed under Ultraviolet-visible Spectorophotometry ⟨*2.24*⟩: the absorbances at 240 nm between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively. The absorption spectrum determined in a 5-cm cell using water as a blank shows a smooth absorption curve between 235 nm and 340 nm.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ethenzamide

Ethoxybenzamide

エテンザミド



$C_9H_{11}NO_2$: 165.19
2-Ethoxybenzamide
[*938-73-8*]

Ethenzamide, when dried, contains not less than 98.0% of ethenzamide ($C_9H_{11}NO_2$).

Description Ethenzamide occurs as white, crystals or crystalline powder.

It is soluble in methanol, in ethanol (95), and in acetone, and practically insoluble in water.

It begins to sublime slightly at about 105°C.

Identification (1) Determine the absorption spectrum of a solution of Ethenzamide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ethenzamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethenzamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Ethenzamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 131 – 134°C

Purity (1) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of Ethenzamide in 30 mL of acetone, add 6 mL of dilute nitric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.7 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone and 6 mL of dilute nitric acid, and dilute with water to make 50 mL (not more than 0.050%).

(2) Sulfate ⟨*1.14*⟩—Dissolve 0.5 g of Ethenzamide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid, and dilute with water to 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone and 1 mL of dilute hydrochloric acid, and dilute with water to 50 mL (not more than 0.048%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ethenzamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic ⟨*1.11*⟩—To 0.40 g of Ethenzamide add 0.3 g of potassium nitrate and 0.5 g of anhydrous sodium carbonate, mix thoroughly, ignite the mixture gradually, and cool. Dissolve the residue in 10 mL of dilute sulfuric acid, and heat the solution until white fumes begin to evolve. After cooling, add water carefully to make 5 mL, use this solution as the test solution, and perform the test (not more than 5 ppm).

(5) Salicylamide—Dissolve 0.20 g of Ethenzamide in 15 mL of diluted ethanol (95) (2 in 3), and add 2 to 3 drops of

dilute iron (III) chloride TS: no purple color develops.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, silica gel, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Ethenzamide and Ethenzamide RS, previously dried, and dissolve each in 70 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using ethanol (95) as the blank.

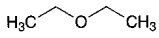
Amount (mg) of ethenzamide ($C_9H_{11}NO_2$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Ethenzamide RS taken

Containers and storage Containers—Well-closed containers.

Ether

エーテル



$C_4H_{10}O$: 74.12
Diethyl ether
[*60-29-7*]

Ether contains not less than 96% and not more than 98% (by specific gravity) of ether ($C_4H_{10}O$).

It contains a small quantity of ethanol and water.

It cannot be used for anesthesia.

Description Ether is a colorless, clear, mobile liquid, having a characteristic odor.

It is miscible with ethanol (95).

It is soluble in water.

It is highly volatile and flammable.

It is slowly oxidized by the action of air and light, with the formation of peroxides.

Its vapor, when mixed with air and ignited, may explode violently.

Boiling point: 35 – 37°C

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.718 – 0.721

Purity **(1)** Foreign odor—Place 10 mL of Ether in an evaporating dish, and allow it to evaporate spontaneously to a volume of about 1 mL: no foreign odor is perceptible. Drop this residue onto a piece of clean, odorless filter paper to evaporate the ether: no foreign odor is perceptible.

(2) Acidity—Place 10 mL of diluted ethanol (95) (4 in 5) and 0.5 mL of phenolphthalein TS in a 50-mL glass-stoppered flask, and add 0.02 mol/L sodium hydroxide VS dropwise to produce a red color which persists after shaking for 30 seconds. Add 25 mL of Ether, stopper the flask, shake gently, and add 0.40 mL of 0.02 mol/L sodium hydroxide VS with shaking: a red color develops.

(3) Aldehyde—Place 10 mL of Ether in a Nessler tube, add 1 mL of potassium hydroxide TS, and allow the mixture to stand for 2 hours, protecting from light, with occasional shaking: no color is produced in the ether layer and the aqueous layer.

(4) Peroxide—Place 10 mL of Ether in a Nessler tube, add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake for 1 minute, then add 1 mL of starch TS, and shake well: no color is produced in the ether layer and in the aqueous layer.

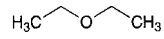
(5) Residue on evaporation—Evaporate 140 mL of Ether, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Containers and storage Containers—Tight containers.

Storage—Without fill up, light-resistant, remote from fire, and not exceeding 25°C.

Anesthetic Ether

麻酔用エーテル



$C_4H_{10}O$: 74.12
Diethyl ether
[*60-29-7*]

Anesthetic Ether contains not less than 96% and not more than 98% (by specific gravity) of ether ($C_4H_{10}O$).

It contains small quantities of ethanol and water. Suitable stabilizers may be added.

It is not to be used for anesthesia if it has been removed from the original container for more than 24 hours.

Description Anesthetic Ether occurs as a colorless, clear, mobile liquid, having a characteristic odor.

It is miscible with ethanol (95).

It is soluble in water.

It is highly volatile and flammable.

It is slowly oxidized by the action of air and light, with the formation of peroxides.

Its vapor, when mixed with air and ignited, may explode violently.

Boiling point: 35 – 37°C

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.718 – 0.721

Purity **(1)** Foreign odor—Place 10 mL of Anesthetic Ether in an evaporating dish, and allow it to evaporate spontaneously to a volume of about 1 mL: no foreign odor is perceptible. Drop this residue onto a piece of clean, odorless filter paper to evaporate the ether: no foreign odor is perceptible.

(2) Acidity—Place 10 mL of diluted ethanol (95) (4 in 5) and 0.5 mL of phenolphthalein TS in a 50-mL glass-stoppered flask, and add 0.02 mol/L sodium hydroxide VS dropwise to produce a red color which persists after shaking for 30 seconds. Add 25 mL of Anesthetic Ether, stopper the flask, shake gently, and add 0.40 mL of 0.02 mol/L sodium hydroxide VS with shaking: a red color develops.

(3) Aldehyde—To 100 mL of water in a 200-mL glass-stoppered flask add 10 mL of Anesthetic Ether and 1 mL of a solution of sodium hydrogen sulfite (1 in 1000), stopper tightly, shake vigorously for 10 seconds, and allow the mixture to stand in a cool place for 30 minutes, protected from light. Add 2 mL of starch TS, and add dropwise 0.01 mol/L iodine VS until a pale blue color develops. Shake with about 2 g of sodium hydrogen carbonate to decolorize the solution, and add 1 mL of diluted 0.01 mol/L iodine VS (9 in 40): a blue color develops. Keep the temperature of the solution below 18°C during the procedure.

(4) Peroxide—Place 10 mL of Anesthetic Ether in a

Nessler tube, add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake occasionally for 1 hour, protecting from light, then add 1 mL of starch TS, and shake well: no color is produced and in the aqueous layer and in the ether layer.

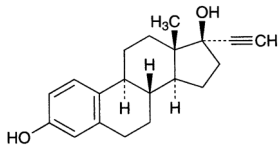
(5) Residue on evaporation—Evaporate 50 mL of Anesthetic Ether, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Containers and storage Containers—Tight containers.

Storage—Without fill up, light-resistant, remote from fire, and not exceeding 25°C.

Ethinylestradiol

エチニルエストラジオール



$C_{20}H_{24}O_2$: 296.40

19-Nor-17α-pregna-1,3,5(10)-triene-20-yne-3,17-diol

[*57-63-6*]

Ethinylestradiol, when dried, contains not less than 98.0% of ethinylestradiol ($C_{20}H_{24}O_2$).

Description Ethinylestradiol occurs as white to pale yellow, crystals or crystalline powder. It is odorless.

It is freely soluble in pyridine and in tetrahydrofuran, soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 2 mg of Ethinylestradiol in 1 mL of a mixture of sulfuric acid and ethanol (95) (1:1): a purplish red color develops with a yellow-green fluorescence. Add carefully 2 mL of water to this solution: the color of the solution changes to red-purple.

(2) Transfer 0.02 g of Ethinylestradiol to a glass-stoppered test tube, dissolve in 10 mL of a solution of potassium hydroxide (1 in 20), add 0.1 g of benzoyl chloride, and shake. Collect the resulting precipitate, recrystallize from methanol, and dry in a desiccator (in vacuum, phosphorus (V) oxide): the precipitate melts ⟨*2.60*⟩ between 200°C and 202°C.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −26 – −31° (after drying, 0.1 g, pyridine, 25 mL, 100 mm).

Melting point ⟨*2.60*⟩ 180 – 186°C or 142 – 146°C

Purity Estrone—Dissolve 5 mg of Ethinylestradiol in 0.5 mL of ethanol (95), and add 0.05 g of 1,3-dinitrobenzene. Add 0.5 mL of freshly prepared dilute potassium hydroxide-ethanol TS, allow to stand in a dark place for 1 hour, and add 10 mL of ethanol (95): the solution has no more color than the following control solution.

Control solution: Proceed in the same manner as mentioned above, omitting Ethinylestradiol.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.2 g of Ethinylestradiol, previously dried, and dissolve in 40 mL of tetrahydrofuran. Add 10 mL of a solution of silver nitrate (1 in 20), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.64 mg of $C_{20}H_{24}O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ethinylestradiol Tablets

エチニルエストラジオール錠

Ethinylestradiol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of ethinylestradiol ($C_{20}H_{24}O_2$: 296.40).

Method of preparation Prepare as directed under Tablets, with Ethinylestradiol.

Identification (1) Evaporate to dryness 5 mL of the sample solution obtained in Assay, and add 2 mL of a mixture of sulfuric acid and ethanol (95) (2:1) to the residue: a light red color with a yellow fluorescence develops. To the solution add carefully 4 mL of water: the color of the solution changes to red-purple.

(2) Evaporate to dryness 10 mL of the sample solution obtained in Assay, add 0.2 mL of acetic acid (31) and 2 mL of phosphoric acid to the residue, and heat on a water bath for 5 minutes: a red color with a yellow-green fluorescence develops.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Ethinylestradiol Tablets in a separator, add 10 mL of 2nd fluid for disintegration test, and shake until the tablet is disintegrated. Add 10 mL of dilute sulfuric acid and 20 mL of chloroform, shake vigorously for 5 minutes, and filter the chloroform layer into a conical flask through filter paper on which 5 g of anhydrous sodium sulfate is placed. Extract the aqueous layer with two 20-mL portions of chloroform, proceed with the extracts in the same manner as before, and combine the filtrates with the previous one. Evaporate gently the combined filtrate on a water bath with the aid of a current of nitrogen, dissolve the residue in exactly 100 mL of methanol, and centrifuge, if necessary. Pipet x mL of the supernatant liquid, add methanol to make exactly V mL of a solution containing about 40 ng of ethinylestradiol ($C_{20}H_{24}O_2$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ethinylestradiol RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in methanol, dilute to a volume containing about 40 ng of ethinylestradiol ($C_{20}H_{24}O_2$) per mL, and use this solution as the standard solution. Pipet 4 mL each of sulfuric acid-methanol TS into three glass-stoppered test tubes, T, S and B, cool in ice, to each tube add exactly 1 mL each of the sample solution, the standard solution and methanol, shake immediately, and allow to stand in a water bath at 30°C for 40 minutes, then allow to stand in a water bath at 20°C for 5 minutes. Perform the test with these solutions as directed under Fluorometry ⟨*2.22*⟩. Determine the fluorescence intensities, F_T, F_S and F_B, of these solutions using the fluorophotometer, at about 460 nm of the excitation and at about 493 nm of the fluorescence.

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)
$= M_S \times (F_T - F_B)/(F_S - F_B) \times V/2500 \times 1/x$

M_S: Amount (mg) of Ethinylestradiol RS taken

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay (i) Chromatographic tube: Pack a pledget of glass wool in the bottom of a tube 25 mm in inside diameter and 300 mm in length, and place 5 g of anhydrous sodium sulfate on the glass wool.

(ii) Chromatographic column: Place 5 g of siliceous earth for chromatography in a 200-mL beaker, soak well in 4 mL of 1 mol/L hydrochloric acid TS, and mix uniformly. Put the siliceous earth into the chromatographic tube in small portions to make 60 to 80 mm in height in proper hardness with a tamping rod.

(iii) Standard solution: Weigh accurately about 10 mg of Ethinylestradiol RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, and add chloroform to make exactly 100 mL.

(iv) Sample: Weigh accurately not less than 20 Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of ethinylestradiol ($C_{20}H_{24}O_2$), place in a 50-mL beaker, add 2 mL of water, shake well, add 3 mL of chloroform, and shake well again. Add 4 g of siliceous earth for chromatography, mix well until the contents do not stick to the inner wall of the beaker, and use the substance as the sample.

(v) Procedure: To the chromatographic column add the sample with a funnel, and pack in proper hardness. Mix well the sample sticking to the beaker with 0.5 g of siliceous earth for chromatography, and place in the chromatographic tube. Wipe off the sample solution sticking to the beaker and the tamping rod with glass wool, and place it in the chromatographic tube. Push down the sample, and press lightly on the chromatographic column to make the height of the column 110 mm to 130 mm. Take 70 mL of chloroform, rinse the inner wall of the chromatographic tube with a portion of the chloroform, and transfer the remaining portion to the chromatographic tube. Collect the effluent solution at a flow rate not more than 0.8 mL per minute. After completing the elution, rinse the lower end of the chromatographic tube with a small quantity of chloroform, add chloroform to make exactly 100 mL, and use this solution as the sample solution. Transfer 6 mL each of the sample solution and standard solution to each separators, and add 20 mL each of isooctane. Add exactly 10 mL of a mixture of sulfuric acid and methanol (7:3), shake vigorously for 5 minutes, allow to stand in a dark place for 15 minutes, and centrifuge. Perform the test with the resulting color solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 6 mL of chloroform in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions obtained from the sample solution and standard solution at 540 nm, respectively.

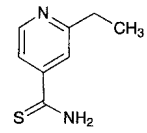
Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)
$= M_S \times A_T/A_S \times 1/20$

M_S: Amount (mg) of Ethinylestradiol RS taken

Containers and storage Coniners—Well-closed containers.

Ethionamide

エチオナミド



$C_8H_{10}N_2S$: 166.24
2-Ethylpyridine-4-carbothioamide
[*536-33-4*]

Ethionamide, when dried, contains not less than 98.5% and not more than 101.0% of ethionamide ($C_8H_{10}N_2S$).

Description Ethionamide occurs as yellow, crystals or crystalline powder, having a characteristic odor.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Ethionamide in methanol (3 in 160,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethionamide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 161 – 165°C

Purity **(1)** Acidity—Dissolve 3.0 g of Ethionamide in 30 mL of methanol by warming, add 90 mL of water, allow to stand in ice water for 1 hour, and filter. To 80 mL of the filtrate add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Ethionamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Ethionamide according to Method 3. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.20 g of Ethionamide in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet exactly 0.2 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, hexane and methanol (6:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the

sample solution is not more intense than the spot obtained with the standard solution (1), and number of the spot other than the principal spot obtained with the sample solution which is more intense than the spot with the standard solution (2) is not more than one.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

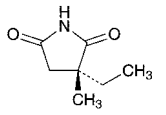
Assay Weigh accurately about 0.3 g of Ethionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of *p*-naphtholbenzein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.62 mg of $C_8H_{10}N_2S$

Containers and storage Containers—Well-closed containers.

Ethosuximide

エトスクシミド



and enantiomer

$C_7H_{11}NO_2$: 141.17
(2*RS*)-2-Ethyl-2-methylsuccinimide
[*77-67-8*]

Ethosuximide contains not less than 98.5% of ethosuximide ($C_7H_{11}NO_2$), calculated on the anhydrous basis.

Description Ethosuximide occurs as a white, paraffin-like solid or powder. It is odorless or has a slight, characteristic odor.

It is very soluble in methanol, in ethanol (95), in diethyl ether, and in *N*,*N*-dimethylformamide, and freely soluble in water.

Melting point: about 48°C

Identification **(1)** To 0.2 g of Ethosuximide add 10 mL of sodium hydroxide TS, and boil: the gas evolved turns a moistened red litmus paper blue.

(2) Dissolve 0.05 g of Ethosuximide in 1 mL of ethanol (95), add 3 drops of a solution of copper (II) acetate monohydrate (1 in 100), warm slightly, and add 1 to 2 drops of sodium hydroxide TS: a purple color is produced.

(3) Determine the absorption spectrum of a solution of Ethosuximide in ethanol (95) (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ethosuximide in 10 mL of water: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—With 1.0 g of Ethosuximide, perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ethosuximide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Ethosuximide, according to Method 1, and perform the test (not more than 2 ppm).

(5) Acid anhydride—Dissolve 0.50 g of Ethosuximide in 1 mL of ethanol (95), add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and allow to stand for 5 minutes. Add 3 mL of water, mix, and allow to stand for 5 minutes: the red to red-purple color of this solution is not more intense than that of the following control solution.

Control solution: Dissolve 70 mg of succinic anhydride in ethanol (95) to make exactly 100 mL. To 1.0 mL of this solution add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and proceed in the same manner.

(6) Cyanide—Dissolve 1.0 g of Ethosuximide in 10 mL of ethanol (95), and add 3 drops of iron (II) sulfate TS, 1 mL of sodium hydroxide TS and 2 to 3 drops of iron (III) chloride TS. Warm gently, and acidify with dilute sulfuric acid: not a blue precipitate and a blue color are produced within 15 minutes.

Water ⟨*2.48*⟩ Not more than 0.5% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ethosuximide, dissolve in 20 mL of *N*,*N*-dimethylformamide, and titrate ⟨*2.50*⟩ with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

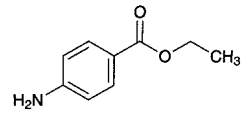
Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 14.12 mg of $C_7H_{11}NO_2$

Containers and storage Containers—Tight containers.

Ethyl Aminobenzoate

Anesthamine
Benzocaine

アミノ安息香酸エチル



$C_9H_{11}NO_2$: 165.19
Ethyl 4-aminobenzoate
[*94-09-7*]

Ethyl Aminobenzoate, when dried, contains not less than 99.0% of ethyl aminobenzoate ($C_9H_{11}NO_2$).

Description Ethyl Aminobenzoate occurs as white, crystals or crystalline powder. It is odorless. It has a slightly bitter taste, numbing the tongue.

It is freely soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It dissolves in dilute hydrochloric acid.

Identification **(1)** Dissolve 0.01 g of Ethyl Aminobenzoate in 1 mL of dilute hydrochloric acid and 4 mL of water.

This solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(2) Dissolve 0.1 g of Ethyl Aminobenzoate in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.

(3) Warm 0.05 g of Ethyl Aminobenzoate with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

Melting point <*2.60*> 89 – 91°C

Purity **(1)** Acidity—Dissolve 1.0 g of Ethyl Aminobenzoate in 10 mL of neutralized ethanol, and add 10 mL of water, 2 drops of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(2) Chloride—Dissolve 0.20 g of Ethyl Aminobenzoate in 5 mL of ethanol (95), add 2 to 3 drops each of dilute nitric acid and of silver nitrate TS: no change occurs immediately.

(3) Heavy metals <*1.07*>—Dissolve 2.0 g of Ethyl Aminobenzoate in 20 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and sufficient ethanol (95) to make 50 mL (not more than 10 ppm).

(4) Readily carbonizable substances <*1.15*>—Perform the test with 0.5 g of Ethyl Aminobenzoate: the solution has no more color than Matching Fluid A.

Loss on drying <*2.41*> Not more than 1.0% (1 g, silica gel, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Ethyl Aminobenzoate, previously dried, dissolve in 10 mL of hydrochloric acid and 70 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), and cool to a temperature below 15°C. Then titrate <*2.50*> with 0.1 mol/L sodium nitrite VS by the potentiometric titration or the amperometric titration.

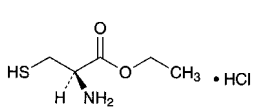
Each mL of 0.1 mol/L sodium nitrite VS
= 16.52 mg of $C_9H_{11}NO_2$

Containers and storage Containers—Well-closed containers.

Ethyl L-Cysteine Hydrochloride

Ethyl Cysteine Hydrochloride

L-エチルシステイン塩酸塩



$C_5H_{11}NO_2S.HCl$: 185.67
Ethyl (2*R*)-2-amino-3-sulfanylpropanoate monohydrochloride
[*868-59-7*]

Ethyl L-Cysteine Hydrochloride, when dried, contains not less than 98.5% of ethyl cysteine hydrochloride ($C_5H_{11}NO_2S.HCl$).

Description Ethyl L-Cysteine Hydrochloride occurs as white, crystals or crystalline powder. It has a characteristic odor, and has a bitter taste at first with a burning aftertaste.

It is very soluble in water, and freely soluble in ethanol (95).

Melting point: about 126°C (with decomposition).

Identification **(1)** Determine the infrared absorption spectrum of Ethyl L-Cysteine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Ethyl L-Cysteine Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −10.0 – −13.0° (after drying, 2.0 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity **(1)** Sulfate <*1.14*>—Perform the test with 0.6 g of Ethyl L-Cysteine Hydrochloride. Prepare the the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Ethyl L-Cysteine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure rapidly. Dissolve 0.05 g each of Ethyl L-Cysteine Hydrochloride and *N*-ethylmaleimide in 5 mL of mobile phase, allow to stand for 30 minutes, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: a peak area from the sample solution with the relative retention time to ethyl L-cysteine-*N*-ethylmaleimide complex from the standard solution being about 0.7 is not larger than the peak area of ethyl L-cysteine-*N*-ethylmaleimide complex from the standard solution. Each area of all peaks other than ethyl L-cysteine-*N*-ethylmaleimide complex and *N*-ethylmaleimide from the sample solution is not larger than 1/3 times the peak area of ethyl L-cysteine *N*-ethylmaleimide complex from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogenphoshate TS and acetonitrile (2:1).

Flow rate: Adjust so that the retention time of ethyl L-cysteine-*N*-ethylmaleimide complex is about 4 minutes.

Selection of column: Dissolve 0.05 g of Ethyl L-Cysteine Hydrochloride, 0.01 g of L-cysteine hydrochloride and 0.05 g of *N*-ethylmaleimide in 25 mL of the mobile phase, and allow to stand for 30 minutes. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of L-cysteine-*N*-ethylmaleimide complex, ethyl L-cysteine-*N*-ethylmaleimide complex and *N*-ethylmaleimide in this order, complete resolution of each component, and the resolution of the peaks of L-cysteine-*N*-ethylmaleimide complex and ethyl L-cysteine-*N*-ethylmaleimide complex being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of ethyl L-cysteine-*N*-ethylmaleimide complex obtained from 2 μL of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About 3 times as long as the retention time of ethyl L-cysteine-*N*-ethylmaleimide complex.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus oxide (V), 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

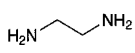
Assay Weigh accurately about 0.25 g of Ethyl L-Cysteine Hydrochloride, previously dried, transfer into a glass-stoppered flask, and dissolve in 10 mL of water previously freshly boiled and cooled to a temperature not exceeding 5°C in a stream of nitrogen. Add exactly 20 mL of 0.05 mol/L iodine VS, previously cooled to a temperature not exceeding 5°C, and allow to stand for 30 seconds, then titrate ⟨*2.50*⟩ with 0.1 mol/L sodium thiosulfate VS, on cooling below 5°C (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 18.57 mg of $C_5H_{11}NO_2S.HCl$

Containers and storage Containers—Tight containers.

Ethylenediamine

エチレンジアミン



$C_2H_8N_2$: 60.10
Ethane-1,2-diamine
[*107-15-3*]

Ethylenediamine contains not less than 97.0% of ethylenediamine ($C_2H_8N_2$).

Description Ethylenediamine is a clear, colorless to pale yellow liquid. It has an ammonia-like odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It has a caustic nature and an irritating property.

It is gradually affected by air.

Specific gravity d_{20}^{20}: about 0.898

Identification **(1)** A solution of Ethylenediamine (1 in 500) is alkaline.

(2) To 2 mL of copper (II) sulfate TS add 2 drops of Ethylenediamine: a blue-purple color develops.

(3) To 0.04 g of Ethylenediamine add 6 drops of benzoyl chloride and 2 mL of a solution of sodium hydroxide (1 in 10), warm for 2 to 3 minutes with occasional shaking, collect the white precipitate formed, and wash with water. Dissolve the precipitate in 8 mL of ethanol (95) by warming, promptly add 8 mL of water, cool, filter the crystals, wash with water, and dry at 105°C for 1 hour: it melts ⟨*2.60*⟩ between 247°C and 251°C.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Place 1.0 g of Ethylenediamine in a porcelain crucible, evaporate to dryness on a water bath, cover loosely, ignite at a low temperature until charred, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Residue on evaporation—Pipet 5 mL of Ethylenediamine, heat on a water bath to dryness, and dry to constant mass at 105°C: the mass of the residue does not exceed 3.0 mg.

Distilling range ⟨*2.57*⟩ 114 – 119°C, not less than 95 vol%.

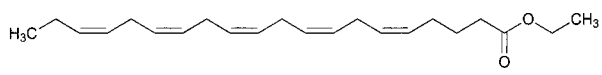
Assay Weigh accurately about 0.7 g of Ethylenediamine in a glass-stoppered conical flask, add 50 mL of water, and titrate ⟨*2.50*⟩ with 1 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS).

Each mL of 1 mol/L hydrochloric acid VS
= 30.05 mg of $C_2H_8N_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and almost well-filled.

Ethyl Icosapentate

イコサペント酸エチル



$C_{22}H_{34}O_2$: 330.50
Ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaenoate
[*86227-47-6*]

Ethyl Icosapentate contains not less than 96.5% and not more than 101.0% of ethyl icosapentate ($C_{22}H_{34}O_2$).

It may contain a suitable antioxidant.

Description Icosapentate is a colorless or pale yellow, clear liquid. It has a faint, characteristic odor.

It is miscible with ethanol (99.5), with acetic acid (100) and with hexane. It is practically insoluble in water and in ethylene glycol.

Identification **(1)** To 20 mg of Ethyl Icosapentate add 3 mL of a solution of potassium hydroxide in ethylene glycol (21 in 100), stopper tightly while passing a current of nitrogen, and heat at 180°C for 15 minutes. After cooling, add methanol to make 100 mL. To 4 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared in the same manner as the sample solution with 3 mL of the solution of potassium hydroxide in ethylene glycol (21 in 100), as a control, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ethyl Icosapentate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethyl Icosapentate as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Ethyl Icosapentate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.481 – 1.491

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.905 – 0.915

Acid value ⟨*1.13*⟩ Not more than 0.5.

Saponification value ⟨*1.13*⟩ 165 – 175

Iodine value ⟨*1.13*⟩ 365 – 395 Perform the test with 20 mg

of Ethyl Icosapentate.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Mix 1.0 g of Ethyl Icosapentate with ethanol (99.5), and add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL. Perform the test with this solution as the test solution.

Control solution: To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Ethyl Icosapentate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—To 0.40 g of Ethyl Icosapentate add hexane to make 50 mL, and use this solution as the sample solution. Perform the test with 1.5 μL of the sample solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of these peaks by the area percentage method: the amount of the peak, having the relative retention time of about 0.53 to ethyl icosapentate, is not more than 0.5%, the amount of each peak, having the relative retention time of about 0.80 and 0.93, is not more than 1.0%, the amount of each peak other than the principal peak and the peak mentioned above is not more than 1.0%, and the total amount of these peaks other than the principal peak is not more than 3.5%.

Operating conditions—

Detector, column, column temperature, carrier gas and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of ethyl icosapentate, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 1 mL of the sample solution add hexane to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add hexane to make exactly 10 mL. Confirm that the peak area of ethyl icosapentate obtained from 1.5 μL of this solution is equivalent to 7 to 13% of that obtained from 1.5 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 1.5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ethyl icosapentate is not more than 2.0%.

(4) Peroxide—Weigh accurately about 1 g of Ethyl Icosapentate, put in a 200-mL glass-stoppered conical flask, add 25 mL of a mixture of acetic acid (100) and chloroform (3:2), and dissolve by gentle shaking. Add 1 mL of saturated potassium iodide solution TS, immediately stopper tightly, shake gently, and allow to stand in a dark place for 10 minutes. Then add 30 mL of water, shake vigorously for 5 to 10 seconds, and titrate ⟨*2.50*⟩ with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 1 mL of starch TS. Calculate the amount of peroxide by the following equation: not more than 2 mEq/kg.

$$\text{Amount (mEq/kg) of peroxide} = V/M \times 10$$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed

M: Amount (g) of Ethyl Icosapentate taken

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Ethyl Icosapentate, and add hexane to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of Ethyl Icosapentate RS, and add hexane to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethyl icosapentate to that of the internal standard.

$$\text{Amount (mg) of ethyl icosapentate } (C_{22}H_{34}O_2) = M_S \times Q_T/Q_S \times 5$$

M_S: Amount (mg) of Ethyl Icosapentate RS taken

Internal standard solution—A solution of methyl docosanate in hexane (1 in 125).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column in 4 mm inside diameter and 1.8 m in length, packed with siliceous earth for gas chromatography (175 to 246 μm in particle diameter), coated with diethylene glycol succinate polyester for gas chromatography in the ratio of 25%.

Column temperature: A constant temperature of about 190°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethyl icosapentate is about 30 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and ethyl icosapentate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethyl icosapentate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Being fully filled, or replacing the air with Nitrogen.

Ethyl Icosapentate Capsules

イコサペント酸エチルカプセル

Ethyl Icosapentate Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of ethyl icosapentate ($C_{22}H_{34}O_2$: 330.50).

Method of preparation Prepare as directed under Capsules, with Ethyl Icosapentate.

Identification Take out the content of Ethyl Icosapentate Capsules, to a quantity of the contents, equivalent to 20 mg of Ethyl Icosapentate, add 3 mL of a solution of potassium hydroxide in ethylene glycol (21 in 100), stopper the vessel tightly while passing a current of nitrogen, and heat at 180°C for 15 minutes. After cooling, add methanol to make 100 mL. To 1 mL of this solution add methanol to make 25 mL, and determine the absorption spectrum of this solution as

directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, obtained by proceeding as above with 3 mL of a solution of potassium hydroxide in ethylene glycol (21 in 100), as a blank: it exhibits maxima between 298 nm and 302 nm, between 311 nm and 315 nm, between 325 nm and 329 nm, and between 343 nm and 347 nm.

Purity Peroxide—Take out the content of Ethyl Icosapentate Capsules. Weigh accurately about 1 g of the content, dissolve in 25 mL of a mixture of acetic acid (100) and isooctane (3:2), replace the air of the inside gently with Nitrogen, then add 1 mL of saturated potassium iodide TS under a current of Nitrogen, stopper immediately and shake gently, and allow to stand in a dark place for 10 minutes. Then, add 30 mL of water, shake vigorously, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. The amount of peroxide calculated by the following formula is not more than 20 mEq/kg.

$$\text{Amount (mEq/kg) of peroxide} = V/M \times 10$$

V: Amount (mL) of 0.01 mol/L sodium thiosulfate VS consumed
M: Amount (g) of Ethyl Icosapentate Capsules taken

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Disintegration <6.09> Perform the test using the disk: it meets the requirement. However, for the preparations in single-dose packages, carry out the test for 10 minutes.

Assay Weigh accurately the mass of an amount of not less than 20 Ethyl Icosapentate Capsules, then open the capsules and take out the contents. Wash the empty capsules with a little amount of hexane, volatilize the hexane by allowing them to stand at the room temperature, and weigh the mass of the total empty capsules accurately. Weigh accurately a portion of the content, equivalent to about 0.4 g of ethyl icosapentate ($C_{22}H_{34}O_2$), add exactly 40 mL of the internal standard solution, then add hexane to make 200 mL, and use this solution as the sample solution. For the preparations in single-dose packages, weigh accurately the mass of the total capsules of not less than 20 packages, and mix them well. Weigh accurately a portion of the capsules, equivalent to about 0.4 g of ethyl icosapentate ($C_{22}H_{34}O_2$), add 15 mL of hexane, then extract the content by opening the capsules. Separate the hexane extract from the residual solids, wash the residues with three 10-mL portions of hexane, combine the washings and the hexane extract, add exactly 40 mL of the internal standard solution, then add hexane to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Ethyl Icosapentate RS, add exactly 5 mL of the internal standard solution, then add hexane to make 25 mL, and use this solution as the standard solution. Perform the test with 4 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethyl icosapentate to that of the internal standard.

$$\text{Amount (mg) of ethyl icosapentate } (C_{22}H_{34}O_2) = M_S \times Q_T/Q_S \times 8$$

M_S: Amount (mg) of Ethyl Icosapentate RS taken

Internal standard solution—A solution of methyl docosanate in hexane (1 in 200).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Ethyl Icosapentate.

System suitability—

System performance: When the procedure is run with 4 μL of the standard solution under the above operating conditions, the internal standard and ethyl icosapentate are eluted in this order with the resolution between these peaks being not less than 3.

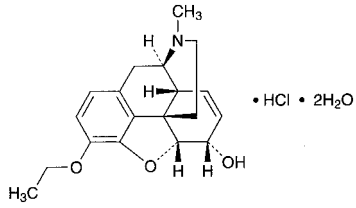
System repeatability: When the test is repeated 6 times with 4 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethyl icosapentate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ethylmorphine Hydrochloride Hydrate

Dionin

エチルモルヒネ塩酸塩水和物



$C_{19}H_{23}NO_3.HCl.2H_2O$: 385.88
(5*R*,6*S*)-4,5-Epoxy-3-ethoxy-17-methyl-7,8-didehydromorphinan-6-ol monohydrochloride dihydrate [*125-30-4*, anhydride]

Ethylmorphine Hydrochloride Hydrate contains not less than 98.0% of ethylmorphine hydrochloride ($C_{19}H_{23}NO_3.HCl$: 349.85), calculated on the anhydrous basis.

Description Ethylmorphine Hydrochloride Hydrate occurs as white to pale yellow, crystals or crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water, soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is affected by light.

Melting point: about 123°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ethylmorphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethylmorphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ethylmorphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: -103 – $-106°$ (0.4 g calcu-

lated on the anhydrous basis, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 0.10 g of Ethylmorphine Hydrochloride Hydrate in 10 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity Related substances—Dissolve 0.20 g of Ethylmorphine Hydrochloride Hydrate in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add diluted ethanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ 8.0 – 10.0% (0.25 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

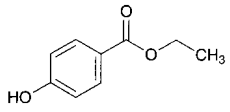
Assay Weigh accurately about 0.5 g of Ethylmorphine Hydrochloride Hydrate, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.99 mg of $C_{19}H_{23}NO_3.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ethyl Parahydroxybenzoate

パラオキシ安息香酸エチル



$C_9H_{10}O_3$: 166.17
Ethyl 4-hydroxybenzoate
[*120-47-8*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Ethyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of ethyl parahydroxybenzoate ($C_9H_{10}O_3$).

◆**Description** Ethyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water.◆

Identification Determine the infrared absorption spectrum of Ethyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Ethyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 115 – 118°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Ethyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

◆**(3)** Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 50 mg of Ethyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of parahydroxybenzoic acid having a relative retention time of about 0.5 to ethyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of ethyl parahydroxybenzoate obtained from the standard solution (0.5%). For the peak area of parahydroxybenzoic acid, multiply its relative response factor, 1.4. Furthermore, the area of the peak other than ethyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of ethyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than ethyl parahydroxybenzoate is not larger than 2 times the peak area of ethyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of ethyl parahydroxybenzoate from the standard solution is excluded (0.1%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of ethyl parahydroxybenzoate.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◆Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ethyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that obtained with 10 μL of the standard solution.◆

◆System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethyl parahydroxybenzoate is not more than 2.0%.◆

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Ethyl Parahydroxybenzoate and Ethyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of ethyl parahydroxybenzoate in each solution.

Amount (mg) of ethyl parahydroxybenzoate ($C_9H_{10}O_3$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Ethyl Parahydroxybenzoate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

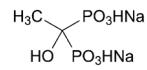
System performance: Dissolve 5 mg each of Ethyl Parahydroxybenzoate, methyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid, methyl parahydroxybenzoate and ethyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid and methyl parahydroxybenzoate to ethyl parahydroxybenzoate are about 0.5 and about 0.8, respectively, and the resolution between the peaks of methyl parahydroxybenzoate and ethyl parahydroxybenzoate is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Etidronate Disodium

エチドロン酸二ナトリウム



$C_2H_6Na_2O_7P_2$: 249.99

Disodium dihydrogen 1-hydroxyethane-1,1-diyldiphosphonate [*7414-83-7*]

Etidronate Disodium, when dried, contains not less than 98.0% and not more than 101.0% of etidronate disodium ($C_2H_6Na_2O_7P_2$).

Description Etidronate Disodium occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

The pH of a solution prepared by dissolving 0.10 g of Etidronate Disodium in 10 mL of water is between 4.4 and 5.4.

It is hygroscopic.

Identification **(1)** To 5 mL of a solution of Etidronate Disodium (1 in 100) add 1 mL of copper (II) sulfate TS, and mix for 10 minutes: a blue precipitate is formed.

(2) Determine the infrared absorption spectrum of Etidronate Disodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Etidronate Disodium (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Etidronate Disodium according to Method 4, and perform the test using the supernatant liquid obtained by centrifuging after addition of 2 mL of dilute acetic acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Etidronate Disodium according to Method 1, and perform the test (not more than 2 ppm).

(3) Phosphite—Weigh accurately about 3.5 g of Etidronate Disodium, dissolve in 100 mL of 0.1 mol/L sodium dihydrogen phosphate TS adjusted the pH to 8.0 with sodium hydroxide TS, add exactly 20 mL of 0.05 mol/L iodine VS, and immediately stopper tightly. Allow to stand in a dark place for 30 minutes, add 1 mL of acetic acid (100), and titrate ⟨*2.50*⟩ the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. The amount of phosphite (NaH_2PO_3) is not more than 1.0%.

Each mL of 0.05 mol/L iodine VS = 5.199 mg of NaH_2PO_3

(4) Methanol—Weigh accurately about 0.5 g of Etidronate Disodium, dissolve in water to make exactly 5 mL, and use this solution as the sample solution. Separately, pipet 1 mL of methanol, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of methanol in each solu-

tion and determine the amount of methanol (CH_4O) by the following equation: not more than 0.1%.

$$\text{Amount (\%) of methanol } (CH_4O) = 1/M \times A_T/A_S \times 1/20 \times 0.79$$

M: Amount (g) of Etidronate Disodium taken
0.79: Density (g/mL) of methanol

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous copolymer beads for gas chromatography (180 – 250 μm in particle diameter).

Column temperature: A constant temperature of about 130°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of methanol is about 2 minutes.

System suitability—

System performance: To 1 mL of methanol and 1 mL of ethanol (99.5) add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. When the procedure is run with 1 μL of this solution under the above operating conditions, methanol and ethanol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methanol is not more than 5.0%.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, 210°C, 2 hours).

Assay Weigh accurately about 0.5 g of Etidronate Disodium, previously dried, and dissolve in water to make exactly 50 mL. Transfer exactly 15 mL of this solution to a chromatographic column of 10 mm in internal diameter containing 5 mL of strongly acidic ion exchange resin for column chromatography (H type), allow to flow at a flow rate of about 1.5 mL per minute, and wash the column with two 25-mL portions of water. Combine the eluate and the washings, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L sodium hydroxide VS} = 12.50 \text{ mg of } C_2H_6Na_2O_7P_2$$

Containers and storage Containers—Tight containers.

Etidronate Disodium Tablets

エチドロン酸二ナトリウム錠

Etidronate Disodium Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of etidronate disodium ($C_2H_6Na_2O_7P_2$: 249.99).

Method of preparation Prepare as directed under Tablets, with Etidronate Disodium.

Identification (1) Shake an amount of powdered Etidronate Disodium Tablets, equivalent to 0.2 g of Etidronate Disodium, with 20 mL of water, and filter. Proceed with the filtrate as directed in the Identification (1) under Etidronate Disodium.

(2) Shake an amount of powdered Etidronate Disodium Tablets, equivalent to 0.4 g of Etidronate Disodium, with 10 mL of water, and filter. Evaporate total amount of the filtrate to dryness under reduced pressure, shake the residue with 15 mL of ethanol (99.5), centrifuge, and dry the precipitate at 150°C for 4 hours. Determine the infrared absorption spectrum of the precipitate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 1170 cm^{-1}, 1056 cm^{-1}, 916 cm^{-1} and 811 cm^{-1}.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Etidronate Disodium Tablets is not less than 85%.

Start the test with 1 tablet of Etidronate Disodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, take exactly V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.22 mg of etidronate disodium ($C_2H_6Na_2O_7P_2$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of etidronate disodium for assay, previously dried at 210°C for 2 hours, and dissolve in water to make exactly 100 mL. Dilute exactly a suitable amount of this solution with water to make solutions so that each mL contains about 0.12 mg, about 0.21 mg and about 0.24 mg of etidronate disodium ($C_2H_6Na_2O_7P_2$), and use these solutions as the standard solutions. Pipet 2 mL each of the sample solution and standard solutions, add exactly 2 mL of a solution of copper (II) sulfate (7 in 10,000) and water to make exactly 10 mL. Determine the absorbances of these solutions at 233 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution prepared by diluting exactly 2 mL of the solution of copper (II) sulfate (7 in 10,000) with water to make exactly 10 mL as the control. From the calibration curve obtained with the standard solutions calculate the concentration of etidronate disodium, C_T, in the sample solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of etidronate disodium } (C_2H_6Na_2O_7P_2) = C_T \times V'/V \times 1/C \times 90$$

C_T: Concentration (μg/mL) of etidronate disodium ($C_2H_6Na_2O_7P_2$) in the sample solution
C: Labeled amount (mg) of etidronate disodium ($C_2H_6Na_2O_7P_2$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Etidronate Disodium Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of etidronate disodium ($C_2H_6Na_2O_7P_2$), add 30 mL of water, shake vigorously for 10 minutes, add water to make exactly 50 mL, and filter. Proceed with exactly 15 mL of the filtrate as directed in the Assay under Etidronate Disodium.

Containers and storage Containers—Tight containers.

Etilefrine Hydrochloride

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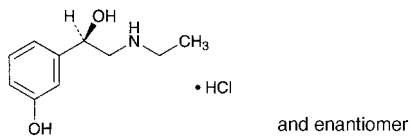


and enantiomer

$C_{10}H_{15}NO_2.HCl$: 217.69
(1*RS*)-2-Ethylamino-1-(3-hydroxyphenyl)ethanol monohydrochloride
[*943-17-9*]

Etilefrine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of etilefrine hydrochloride ($C_{10}H_{15}NO_2.HCl$).

Description Etilefrine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in ethanol (99.5), and sparingly soluble in acetic acid (100).

It is gradually colored to yellow-brown by light.

A solution of Etilefrine Hydrochloride (1 in 20) shows no optical rotation.

Identification **(1)** Dissolve 5 mg of Etilefrine Hydrochloride in 100 mL of diluted hydrochloric acid (1 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etilefrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Etilefrine Hydrochloride (1 in 1000) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Melting point <*2.60*> 118 – 122°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Etilefrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—To 10 mL of a solution of Etilefrine Hydrochloride (1 in 50) add 0.1 mL of methyl red TS for acid or alkali test and 0.2 mL of 0.01 mol/L sodium hydroxide VS: a yellow color develops, and the necessary volume of 0.01 mol/L hydrochloric acid VS to change the color to red is not more than 0.4 mL.

(3) Sulfate <*1.14*>—Perform the test with 0.85 g of Etilefrine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.020%).

(4) Heavy metals <*1.07*>—Dissolve 1.0 g of Etilefrine Hydrochloride in 30 mL of water and 2 mL of acetic acid (100), adjust with sodium hydroxide TS to a pH of 3.3, add water to make 50 mL, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Etilefrine Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correctoin.

Each mL of 0.1 mol/L perchloric acid VS
= 21.77 mg of $C_{10}H_{15}NO_2.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Etilefrine Hydrochloride Tablets

エチレフリン塩酸塩錠

Etilefrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of etilefrine hydrochloride ($C_{10}H_{15}NO_2.HCl$: 217.69).

Method of preparation Prepare as directed under Tablets, with Etilefrine Hydrochloride.

Identification To a quantity of powdered Etilefrine Hydrochloride Tablets, equivalent to 5 mg of Etilefrine Hydrochloride, add 60 mL of diluted hydrochloric acid (1 in 1000), shake well, add 40 mL of diluted hydrochloric acid (1 in 1000), and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using diluted hydrochloric acid (1 in 1000) as the blank: it exhibits a maximum between 271 nm and 275 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Etilefrine Hydrochloride Tablets add 60 mL of diluted hydrochloric acid (1 in 1000), and proceed as directed in the Assay.

Amount (mg) of etilefrine hydrochloride ($C_{10}H_{15}NO_2.HCl$)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of etilefrine hydrochloride for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Etilefrine Hydrochloride Tablets is not less than 70%.

Start the test with 1 tablet of Etilefrine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5 μg of etilefrine hydrochloride ($C_{10}H_{15}NO_2.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of etilefrine hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of etilefrine in each solution.

Dissolution rate (%) with respect to the labeled amount of etilefrine hydrochloride ($C_{10}H_{15}NO_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of etilefrine hydrochloride for assay taken

C: Labeled amount (mg) of etilefrine hydrochloride ($C_{10}H_{15}NO_2$.HCl) in 1 tablet.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of etilefrine are not less than 8000 and 0.9 – 1.2, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etilefrine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Etilefrine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of etilefrine hydrochloride ($C_{10}H_{15}NO_2$.HCl), add 60 mL of diluted hydrochloric acid (1 in 1000), shake for 10 minutes, add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of etilefrine hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in diluted hydrochloric acid (1 in 1000) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as direct under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of etilefrine in each solution.

Amount (mg) of etilefrine hydrochloride ($C_{10}H_{15}NO_2$.HCl)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of etilefrine hydrochloride for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 5 g of sodium lauryl sulfate in 940 mL of water and 500 mL of acetonitrile, and adjust the pH to 2.3 with phosphoric acid.

Flow rate: Adjust so that the retention time of etilefrine is about 6 minutes.

System suitability—

System performance: Dissolve 4 mg of bamethan sulfate and 4 mg of etilefrine hydrochloride in the mobile phase to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, etilefrine and bamethan are eluted in this order with the resolution between these peaks being not less than 5.

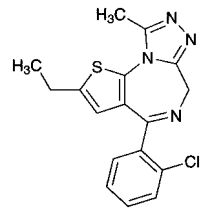
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etilefrine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Etizolam

エチゾラム



$C_{17}H_{15}ClN_4S$: 342.85

4-(2-Chlorophenyl)-2-ethyl-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine

[*40054-69-1*]

Etizolam, when dried, contains not less than 98.5% and not more than 101.0% of etizolam ($C_{17}H_{15}ClN_4S$).

Description Etizolam occurs as a white to pale yellowish white crystalline powder.

It is soluble in ethanol (99.5), sparingly soluble in acetonitrile and in acetic anhydride, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Etizolam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etizolam as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 146 – 149°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Etizolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Etizolam in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 20 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than etizolam obtained from the sample solution is not larger than the peak area of etizolam obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust so that the retention time of etizolam is about 6 minutes.

Time span of measurement: About 5 times as long as the retention time of etizolam, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of etizolam obtained from 10 μL of this solution is equivalent to 8 to 12% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 0.02 g each of Etizolam and ethyl parahydroxybenzoate in the mobile phase to make 50 mL. To 1 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ethyl parahydroxybenzoate and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Etizolam, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). The end point is the second equivalent point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.14 mg of $C_{17}H_{15}ClN_4S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Etizolam Fine Granules

エチゾラム細粒

Etizolam Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of etizolam ($C_{17}H_{15}ClN_4S$: 342.85).

Method of preparation Prepare as directed under Granules, with Etizolam.

Identification (1) To a quantity of powdered Etizolam Fine Granules, equivalent to 5 mg of Etizolam, add 10 mL of methanol, shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Evaporate the filtrate to dryness on a water bath, cool, and then dissolve the residue in 2 mL of sulfuric acid. The solution gives off a light yellow-green fluorescent when exposed to ultraviolet light (main wavelength: 365 nm).

(2) To a quantity of powdered Etizolam Fine Granules, equivalent to 1 mg of Etizolam, add 80 mL of 0.1 mol/L hydrochloric acid TS, shake, and then filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits absorption maxima between 249 nm and 253 nm, and between 292 nm and 296 nm, when perform the measurement within 10 minutes.

Uniformity of dosage units ⟨*6.02*⟩ The Granules in single-dose packages meet the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Etizolam Fine Granules is not less than 75%.

Start the test with an accurately weighed amount of Etizolam Fine Granules, equivalent to about 1 mg of etizolam ($C_{17}H_{15}ClN_4S$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of filtrate, pipet 2 mL of the subsequent filtrate, add exactly 2 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of etizolam in each solution.

Dissolution rate (%) with respect to the labeled amount of etizolam ($C_{17}H_{15}ClN_4S$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 18/5$

M_S: Amount (mg) of etizolam for assay taken
M_T: Amount (g) of Etizolam Fine Granules taken
C: Labeled amount (mg) of etizolam ($C_{17}H_{15}ClN_4S$) in 1 g

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 243 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of etizolam is about 7 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of etizolam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2.0%.

Assay Weigh accurately an amount of powdered Etizolam Fine Granules, equivalent to about 4 mg of etizolam ($C_{17}H_{15}ClN_4S$), add 30 mL of water, and stir. Add 60 mL of methanol, stir for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add

diluted methanol (7 in 10) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, and add diluted methanol (7 in 10) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (7 in 10) to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of etizolam to that of the internal standard.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = M_S \times Q_T/Q_S \times 1/25$$

M_S: Amount (mg) of etizolam for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust so that the retention time of etizolam is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of etizolam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Etizolam Tablets

エチゾラム錠

Etizolam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of etizolam ($C_{17}H_{15}ClN_4S$: 342.85).

Method of preparation Prepare as directed under Tablets, with Etizolam.

Identification **(1)** To a quantity of powdered Etizolam Tablets, equivalent to 5 mg of Etizolam, add 10 mL of methanol, shake, and filter. Evaporate the filtrate to dryness on a water bath, and dissolve the residue in 2 mL of sulfuric acid. The solution gives off a light yellow-green fluorescence when exposed to ultraviolet light (main wavelength: 365 nm).

(2) To a quantity of powdered Etizolam Tablets, equivalent to 1 mg of Etizolam, add 80 mL of 0.1 mol/L hydrochloric acid TS, shake, and then filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of this filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits absorption maxima between 249 nm and 253 nm, and between 292 nm and 296 nm when perform the measurement within 10 minutes.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Etizolam Tablets add 2.5 mL of water, and stir until the tablet is disintegrated. Add 20 mL of methanol, stir for 20 minutes, add methanol to make exactly 25 mL, and centrifuge. Pipet V mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL so that each mL contains about 8 μg of etizolam ($C_{17}H_{15}ClN_4S$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = M_S \times Q_T/Q_S \times 1/V \times 1/20$$

M_S: Amount (mg) of etizolam for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (9 in 10) (1 in 10,000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Etizolam Tablets is not less than 70%.

Start the test with 1 tablet of Etizolam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.28 μg of etizolam ($C_{17}H_{15}ClN_4S$). Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of etizolam for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of etizolam in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of etizolam } (C_{17}H_{15}ClN_4S) = M_S \times A_T/A_S \times V'/V \times 1/C \times 9/10$$

M_S: Amount (mg) of etizolam for assay taken

C: Labeled amount (mg) of etizolam ($C_{17}H_{15}ClN_4S$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 243 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

30°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of etizolam is about 7 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of etizolam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2.0%.

Assay To 20 Etizolam Tablets add 50 mL of water, and stir until they disintegrate. Add 400 mL of methanol, stir for 20 minutes, add methanol to make exactly 500 mL, and centrifuge. Pipet an amount of the supernatant liquid, equivalent to about 0.2 mg of etizolam ($C_{17}H_{15}ClN_4S$), add exactly 2 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 100 mg of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (9 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, and add diluted methanol (9 in 10) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of etizolam to that of the internal standard.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = M_S \times Q_T/Q_S \times 1/500$$

M_S: Amount (mg) of etizolam for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (9 in 10) (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust so that the retention time of etizolam is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

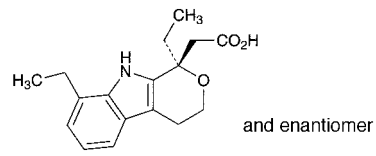
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of etizolam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Etodolac

エトドラク



and enantiomer

$C_{17}H_{21}NO_3$: 287.35

2-[(1*RS*)-1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid

[*41340-25-4*]

Etodolac, when dried, contains not less than 98.5% and not more than 101.0% of etodolac ($C_{17}H_{21}NO_3$).

Description Etodolac occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Etodolac in methanol (1 in 50) shows no optical rotation.

Melting point: about 147°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Etodolac in ethanol (99.5) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etodolac as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Etodolac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.5 g of Etodolac in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 4 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Previously develop a plate of silica gel with fluorescent indicator for thin-layer chromatography in a developing container containing 2 cm depth of a solution of L-ascorbic acid in a mixture of methanol and water (4:1) (1 in 200 mL) to the distance of 3 cm, and air-dry for 30 minutes. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on the plate 2.5 cm away from the bottom of the plate, then immediately develop with a mixture of toluene, ethanol (95) and acetic acid (100) (140:60:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot obtained with the sample solution is not more intense than the spot obtained with the standard solution (1), and the number of spots which are more intense than the

spot with the standard solution (2) is not more than 2.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

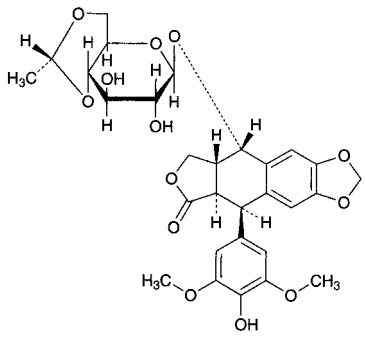
Assay Weigh accurately about 0.3 g of Etodolac, previously dried, dissolve in 50 mL of ethanol (99.5), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 28.74 mg of $C_{17}H_{21}NO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Etoposide

エトポシド



$C_{29}H_{32}O_{13}$: 588.56
(5*R*,5a*R*,8a*R*,9*S*)-9-{[4,6-*O*-(1*R*)-Ethylidene-*β*-D-glucopyranosyl]oxy}5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydrofuro[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one
[*33419-42-0*]

Etoposide contains not less than 98.0% and not more than 102.0% of etoposide ($C_{29}H_{32}O_{13}$), calculated on the anhydrous basis.

Description Etoposide occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Melting point: about 260°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Etoposide in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Etoposide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etoposide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Etoposide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −100 – −105° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Etoposide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Etoposide in 10 mL of methanol, add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than etoposide with the sample solution is not larger than 1/5 times the peak area of etoposide with the standard solution, and the total area of the peaks other than etoposide is not larger than 1/2 times the peak area of etoposide with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of etoposide, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of etoposide obtained with 50 μL of this solution is equivalent to 7 to 13% of that obtained with 50 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etoposide is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Etoposide and Etoposide RS (previously determined the water ⟨*2.48*⟩ in the same manner as Etoposide) dissolve separately in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of etoposide to that of the internal standard.

Amount (mg) of etoposide ($C_{29}H_{32}O_{13}$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Etoposide RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of 2,6-dichlorophenol in methanol (3 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 290 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with phenylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 6.44 g of sodium sulfate decahy-

drate in diluted acetic acid (100) (1 in 100) to make 1000 mL, and add 250 mL of acetonitrile.

Flow rate: Adjust so that the retention time of etoposide is about 20 minutes.

System suitability—

System performance: Dissolve 10 mg of Etoposide in 2 mL of methanol, add 8 mL of the mobile phase, and mix well. Add 0.1 mL of diluted acetic acid (100) (1 in 25) and 0.1 mL of phenolphthalein TS, and add sodium hydroxide TS until the color of the solution changes to faintly red. After allowing to stand for 15 minutes, add 0.1 mL of diluted acetic acid (100) (1 in 25). When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of etoposide and the peak having the relative retention time of about 1.3 to etoposide is not less than 3.

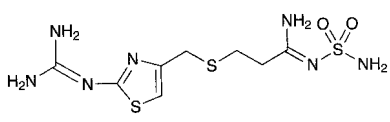
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of etoposide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Famotidine

ファモチジン



$C_8H_{15}N_7O_2S_3$: 337.45

N-Aminosulfonyl-3-{[2-(diaminomethyleneamino)-1,3-thiazol-4-yl]methylsulfanyl}propanimidamide

[*76824-35-6*]

Famotidine, when dried, contains not less than 98.5% of famotidine ($C_8H_{15}N_7O_2S_3$).

Description Famotidine occurs as white to yellowish white crystals.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in 0.5 mol/L hydrochloric acid TS.

It is gradually colored by light.

Melting point: about 164°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Famotidine in 0.05 mol/L potassium dihydrogen phosphate TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Famotidine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Famotidine in 10 mL of 0.5 mol/L hydrochloric acid TS: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Famotidine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Famotidine in 10 mL of acetic acid (100), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetic acid (100) to make exactly 100 mL. Pipet 1 mL, 2 mL and 3 mL of this solution, add acetic acid (100) to make exactly 10 mL, respectively, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel (5 to 7 μm) with fluorescent indicator for thin-layer chromatography, and dry in a stream of nitrogen. Develop the plate with a mixture of ethyl acetate, methanol, toluene and ammonia solution (28) (40:25:20:2) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution (3). Total intensity of the spots other than the principal spot and the spot of the starting point from the sample solution is not more than 0.5% calculated on the basis of intensities of the spots from the standard solution (1) and the standard solution (2).

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Famotidine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.87 mg of $C_8H_{15}N_7O_2S_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Famotidine Injection

ファモチジン注射液

Famotidine Injection is an aqueous injection.

It contains not less than 92.0% and not more than 108.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$: 337.45).

Method of preparation Prepare as directed under Injections, with Famotidine.

Description Famotidine Injection is a colorless or light yellow, clear liquid.

Identification To an amount of Famotidine Injection, equivalent to 10 mg of Famotidine, add water to make 100 mL. Run 1 mL of this solution on a column prepared by filling about 1 cm inside diameter chromatography tube with about 0.4 g of 55 – 105 μm octadecylsilanized silica gel for pretreatment. Wash the column with 15 mL of water, followed by elution with 5 mL of methanol. To the eluate add methanol to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 285 nm and 289 nm.

Osmotic pressure ratio Being specified separately when the

drug is granted approval based on the Law.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Related substances—To an exact amount of Famotidine Injection, equivalent to 25 mg of Famotidine, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of famotidine for assay, dissolve in methanol, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak area of each solution by the automatic integration method, and calculate the amounts of the related substances by the following equation: the amounts of related substances, having the relative retention time about 1.3 and about 1.5 to famotidine are not more than 3.0% respectively, and the amount of other related substances except the above substances is not more than 0.5%, and the total amount of the related substances is not more than 5.0%.

$$\text{Amount (\%) of related substance} = M_S \times A_T/A_S \times 1/10$$
$$\text{Total amount (\%) of related substances} = M_S \times \Sigma A_T/A_S \times 1/10$$

M_S: Amount (mg) of famotidine for assay taken
A_S: Peak area of famotidine in the standard solution
A_T: Peak area of related substances in the sample solution
ΣA_T: Total peak area of the related substances in the sample solution

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 1.74 g of sodium 1-pentane sulfonate in 900 mL of water, adjust to pH 4.0 with diluted acetic acid (100) (1 in 10), and add water to make 1000 mL. To 840 mL of this solution add 80 mL of methanol and 40 mL of acetonitrile.

Flow rate: Adjust so that the retention time of famotidine is about 17 minutes.

Time span of measurement: About 4 times as long as the retention time of famotidine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 50 mL. Confirm that the peak area of famotidine obtained with 20 μL of this solution is equivalent to 8 to 12% of that obtained with 20 μL of the standard solution.

System performance: To 20 mg of famotidine for assay add 2 mL of a solution of methyl parahydroxybenzoate in acetonitrile (1 in 500), and add methanol to make 20 mL. To 5 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, famotidine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 19.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operations conditions, the relative standard deviation of the peak area of famotidine is not more than 2.0%.

Bacterial endotoxins <*4.01*> Less than 15 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method I: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Famotidine Injection, equivalent to about 25 mg of famotidine ($C_8H_{15}N_7O_2S_3$), add exactly 2.5 mL of the internal standard solution, and add the mobile phase to make 50 mL. Pipet 10 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol, add exactly 5 mL of the internal standard solution, and add methanol to make 50 mL. Pipet 5 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratios, Q_T and Q_S of the peak area of famotidine to that of the internal standard.

$$\text{Amount (mg) of famotidine } (C_8H_{15}N_7O_2S_3) = M_S \times Q_T/Q_S \times 1/2$$

M_S: Amount (mg) of famotidine for assay taken

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 500).

Operating conditions—

Ditector: An ultraviolet absorption photometer (Wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.74 g of sodium 1-pentane sulfonate in 900 mL of water, adjust to pH 4.0 with diluted acetic acid (100) (1 in 10), and add water to make 1000 mL. To 750 mL of this solution add 200 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust so that the retention time of famotidine is about 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 26.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Famotidine for Injection

注射用ファモチジン

Famotidine for Injection is a preparation for injection which is dissolved before use.

It contains not less than 94.0% and not more than 106.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$: 337.45).

Method of preparation Prepare as directed under Injection, with Famotidine.

Description Famotidine for Injection occurs as white, porous masses or powder.

Identification Dissolve an amount of Famotidine for Injection, equivalent to 0.01 g of Famotidine, in 50 mL of 0.05 mol/L potassium dihydrogen phosphate TS. To 5 mL of this solution add 0.05 mol/L potassium dihydrogen phosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 263 nm and 267 nm.

pH <*2.54*> Dissolve an amount of Famotidine for Injection, equivalent to 0.02 g of Famotidine, in 1 mL of water: the pH of this solution is between 4.9 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Famotidine for Injection, equivalent to 0.02 g of Famotidine, in 1 mL of water: the solution is clear and colorless.

(2) Related substances—Take a number of Famotidine for Injection, equivalent to about 0.1 g of famotidine ($C_8H_{15}N_7O_2S_3$), dissolve each content in water, wash the inside of the container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than famotidine from the sample solution is not larger than peak area of famotidine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of famotidine, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 2 mL of the standard solution add the water to make exactly 20 mL. Confirm that the peak area of famotidine obtained from 5 μL of this solution is equivalent to 8 to 12% of that obtained from 5 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of famotidine is not more than 2.0%.

Water <*2.48*> Not more than 1.5% (0.1 g, coulometric titration).

Bacterial endotoxins <*4.01*> Not more than 15 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take a number of Famotidine for Injection, equivalent to about 0.1 g of famotidine ($C_8H_{15}N_7O_2S_3$), dissolve each content in water, wash the inside of each container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of famotidine to that of the internal standard.

$$\text{Amount (mg) of famotidine } (C_8H_{15}N_7O_2S_3) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of famotidine for assay taken

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust so that the retention time of famotidine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Famotidine Powder

ファモチジン散

Famotidine Powder contains not less than 94.0% and not more than 106.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$: 337.45).

Method of preparation Prepare as directed under Granules or Powders, with Famotidine.

Identification Weigh a portion of Famotidine Powder, equivalent to 0.01 g of Famotidine, add 50 mL of 0.05 mol/L potassium dihydrogen phosphate TS, shake well, and centrifuge. To 5 mL of the supernatant liquid add 0.05 mol/L potassium dihydrogen phosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 263 nm and 267 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Famotidine Powder in single-dose packages meets the requirement of the Content uniformity test.

Take out the total amount of the content of 1 package of Famotidine Powder, add 10 mL of water per 10 mg of famotidine ($C_8H_{15}N_7O_2S_3$), shake well, add 10 mL of methanol, shake well, add methanol to make exactly V mL so that each mL contains about 0.4 mg of famotidine ($C_8H_{15}N_7O_2S_3$), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$)
$= M_S \times Q_T/Q_S \times V/250$

M_S: Amount (mg) of famotidine for assay taken

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rates in 15 minutes of a 20-mg/g powder and a 100-mg/g powder are not less than 80% and not less than 85%, respectively.

Start the test with an accurately weighed amount of Famotidine Powder, equivalent to about 20 mg of famotidine ($C_8H_{15}N_7O_2S_3$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of famotidine for assay, previously dried in vacuum on phosphorus (V) oxide at 80°C for 4 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 266 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 45$

M_S: Amount (mg) of famotidine for assay taken
M_T: Amount (g) of Famotidine Powder taken
C: Labeled amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$) in 1 g

Assay Weigh accurately a portion of Famotidine Powder, equivalent to about 20 mg of famotidine ($C_8H_{15}N_7O_2S_3$), add 20 mL of water, and shake well. Add 20 mL of methanol, then shake well, add methanol to make exactly 50 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of famotidine to that of the internal standard.

Amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of famotidine for assay taken

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust so that the retention time of famotidine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Famotidine Tablets

ファモチジン錠

Famotidine Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$: 337.45).

Method of preparation Prepare as directed under Tablets, with Famotidine.

Identification Weigh a portion of powdered Famotidine Tablets, equivalent to 0.01 g of Famotidine, add 50 mL of 0.05 mol/L potassium dihydrogen phosphate TS, shake well, and centrifuge. To 5 mL of the supernatant liquid add 0.05 mol/L potassium dihydrogen phosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 263 nm and 267 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Famotidine Tablets add 2 mL of water, shake to disintegrate, then add a suitable amount of methanol, and shake well. Add methanol to make exactly V mL of a solution containing about 0.2 mg of famotidine ($C_8H_{15}N_7O_2S_3$) per mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the operating conditions described in the Assay, and calculate the ratios, Q_T and Q_S, of the peak area of famotidine to that of the internal standard.

Amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$)
$= M_S \times Q_T/Q_S \times V/500$

M_S: Amount (mg) of famotidine for assay taken

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Take a number of Famotidine Tablets, equivalent to 0.2 g of famotidine ($C_8H_{15}N_7O_2S_3$), add 50 mL of water, and disintegrate by shaking well. Add 100 mL of methanol, then shake well, add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of famotidine to that of the internal standard.

Amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of famotidine for assay taken

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust so that the retention time of famotidine is about 6 minutes.

System suitability—

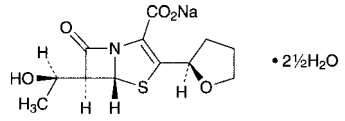
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Faropenem Sodium Hydrate

ファロペネムナトリウム水和物



$C_{12}H_{14}NNaO_5S.2\frac{1}{2}H_2O$: 352.34
Monosodium (5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-7-oxo-3-[(2*R*)-tetrahydrofuran-2-yl]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate hemipentahydrate
[*122547-49-3*, anhydride]

Faropenem Sodium Hydrate contains not less than 870 μg (potency) and not more than 943 μg (potency) per mg, calculated on the anhydrous basis. The potency of Faropenem Sodium Hydrate is expressed as mass (potency) of faropenem ($C_{12}H_{15}NO_5S$: 285.32).

Description Faropenem Sodium Hydrate occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 5 mg of Faropenem Sodium

Hydrate in 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown to brown color develops.

(2) Determine the absorption spectra of solutions of Faropenem Sodium Hydrate and Faropenem Sodium RS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Faropenem Sodium Hydrate and Faropenem Sodium RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +145 – +150° (0.5 g calculated as the anhydrous basis, water, 50 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Faropenem Sodium Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve a quantity of Faropenem Sodium Hydrate equivalent to 0.10 g (potency) in 200 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the epimer, having the relative retention time of about 1.1 to faropenem, obtained from the sample solution is not larger than 3/10 times the peak area of faropenem obtained from the standard solution, and the total area of the peaks other than faropenem from the sample solution is not larger than 1/2 times the peak area of faropenem from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Time span of measurement: About 6 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 2.0%.

Water <*2.48*> Not less than 12.6% and not more than 13.1% (20 mg, coulometric titration).

Assay Weigh accurately an amount of Faropenem Sodium Hydrate and Faropenem Sodium RS, equivalent to about 25 mg (potency), add exactly 10 mL each of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of faropenem to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of faropenem } (C_{12}H_{15}NO_5S) = M_S \times Q_T/Q_S \times 1000$$

M_S: amount [mg (potency)] of Faropenem Sodium RS taken

Internal standard solution—Dissolve 0.5 g of *m*-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 305 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.8 g of potassium dihydrogen phosphate, 5.4 g of disodium hydrogen phosphate dodecahydrate and 1.0 g of tetra *n*-butyl ammonium bromide in water to make 1000 mL. To 870 mL of this solution add 130 mL of acetonitrile.

Flow rate: Adjust so that the retention time of faropenem is about 11 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of faropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Faropenem Sodium for Syrup

シロップ用ファロペネムナトリウム

Faropenem Sodium for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 93.0% and not more than 106.0% of the labeled potency of faropenem ($C_{12}H_{15}NO_5S$: 285.32).

Method of preparation Prepare as directed under Preparations for Syrups, with Faropenem Sodium Hydrate.

Identification Dissolve an amount of powdered Faropenem Sodium for Syrup, equivalent to 25 mg (potency) of Faropenem Sodium Hydrate, in water to make 50 mL. To 5 mL of this solution add water to make 50 mL, filter, if necessary, and determine the absorption spectrum of the solution so obtained as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 254 nm and 258

nm, and between 304 nm and 308 nm.

Purity Related substances—Powder Faropenem Sodium for Syrup, if necessary. To a part of the powder, equivalent to about 25 mg (potency) of Faropenem Sodium Hydrate, add about 10 mL of water, shake well, then add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak of cleaved derivative, having the relative retention time of about 0.71 to faropenem, obtained from the sample solution is not larger than 1.5 times the peak area of faropenem obtained from the standard solution, and the total area of the peaks other than faropenem from the sample solution is not larger than 2 times the peak area of faropenem from the standard solution. For the area of the peak, having the relative retention time of about 0.71 to faropenem, multiply its relative response factor 0.37.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 6.12 g of potassium dihydrogen phosphate, 1.79 g of disodium hydrogen phosphate dodecahydrate and 1.61 g of tetra *n*-butylammonium bromide in water to make 1000 mL.

Mobile phase B: A mixture of the mobile phase A and acetonitrile (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 54	84 → 30	16 → 70

Flow rate: 1.5 mL per minute.

Time span of measurement: 2.5 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 3.0%.

Water <2.48> Not less than 1.5% and not more than 2.1% (80 mg, coulometric titration).

Uniformity of dosage units <6.02> Faropenem Sodium for Syrup in single-dose packages meet the requirement of the Mass variation test.

Assay Powder, if necessary, and weigh accurately an amount of Faropenem Sodium for Syrup, equivalent to about 25 mg (potency) of faropenem ($C_{12}H_{15}NO_5S$), add exactly 10 mL of the internal standard solution and a suitable amount of water, shake well, and add water to make 50 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium RS, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Preceed as directed in the Assay under Faropenem Sodium Hydrate.

$$\text{Amount [mg (potency)] of faropenem } (C_{12}H_{15}NO_5S) = M_S \times Q_T/Q_S$$

M_S: Amount [mg (potency)] of Faropenem Sodium RS taken

Internal standard solution—Dissolve 0.5 g of *m*-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Faropenem Sodium Tablets

ファロペネムナトリウム錠

Faropenem Sodium Tablets contain not less than 94.0% and not more than 106.0% of the labeled potency of faropenem ($C_{12}H_{15}NO_5S$: 285.32).

Method of preparation Prepare as directed under Tablets, with Faropenem Sodium Hydrate.

Identification To powdered Faropenem Sodium Tablets, equivalent to 70 mg (potency) of Faropenem Sodium Hydrate, add water to make 100 mL. To 5 mL of this solution add water to make 100 mL, filter, if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 254 nm and 258 nm and between 304 nm and 308 nm.

Purity Related substances—Powder not less than 5 Faropenem Sodium Tablets. To a part of the powder, equivalent to about 25 mg (potency) of Faropenem Sodium Hydrate, add about 10 mL of water, shake well, then add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak of cleaved derivative, having the relative retention time of about 0.71 to faropenem, obtained from the sample solution is not larger than 1.5 times the peak area of faropenem obtained from the standard solution, and the total area of the peaks other than faropenem from the sample solution is not larger than 2.5 times the peak area of faropenem from the

standard solution. For the area of the peak, having the relative retention time of about 0.71 to faropenem, multiply its relative response factor 0.37.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 6.12 g of potassium dihydrogen phosphate, 1.79 g of disodium hydrogen phosphate dodecahydrate and 1.61 g of tetra *n*-butylammonium bromide in water to make 1000 mL.

Mobile phase B: A mixture of the mobile phase A and acetonitrile (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 54	84 → 30	16 → 70

Flow rate: 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 3.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Faropenem Sodium Tablets add 130 mL of water, shake vigorously until the tablets are disintegrated, and add water to make exactly V mL so that each mL contains about 1 mg (potency) of Faropenem Sodium Hydrate. Pipet 5 mL of this solution, add water to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium RS, and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T275}, A_{T305}, A_{T354}, A_{S275}, A_{S305} and A_{S354}, of the sample solution and standard solution at 275 nm, 305 nm and 354 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and calculate A_T and A_S, using the following equations.

$$A_T = A_{T305} - (49 \times A_{T275} + 30 \times A_{T354})/79$$

$$A_S = A_{S305} - (49 \times A_{S275} + 30 \times A_{S354})/79$$

Amount [mg (potency)] of faropenem ($C_{12}H_{15}NO_5S$) $= M_S \times A_T/A_S \times V/25$

M_S: Amount [mg (potency)] of Faropenem Sodium RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Faropenem Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Faropenem Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg (potency) of Faropenem Sodium Hydrate, and use this solution as the sample solution. Separately, weigh accurately an amount of Faropenem Sodium RS, equivalent to about 18 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 306 nm.

Dissolution rate (%) with respect to the labeled amount of faropenem ($C_{12}H_{15}NO_5S$) $= M_S \times A_T/A_S \times V'/V \times 1/C \times 225$

M_S: Amount [mg (potency)] of Faropenem Sodium RS taken

C: Labeled amount [mg (potency)] of faropenem ($C_{12}H_{15}NO_5S$) in 1 tablet

Assay Weigh accurately the mass of not less than 5 Faropenem Sodium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg (potency) of faropenem ($C_{12}H_{15}NO_5S$), add exactly 10 mL of the internal standard solution, shake well, and add water to make 50 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium RS, add exactly 10 mL of the internal standarad solution and water to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Faropenem Sodium Hydrate.

Amount [mg (potency)] of faropenem ($C_{12}H_{15}NO_5S$) $= M_S \times Q_T/Q_S$

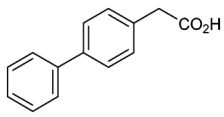
M_S: Amount [mg (potency)] of Faropenem Sodium RS taken

Internal standard solution—Dissolve 0.5 g of *m*-hydorxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Containers and storage Containers—Tight containers.

Felbinac

フェルビナク



$C_{14}H_{12}O_2$: 212.24
Biphenyl-4-ylacetic acid
[*5728-52-9*]

Felbinac, when dried, contains not less than 98.5% and not more than 101.0% of felbinac ($C_{14}H_{12}O_2$).

Description Felbinac occurs as white to pale yellowish white, crystals or crystalline powder.

It is soluble in methanol and in acetone, sparingly soluble in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Felbinac in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Felbinac as directed in the potassium bromide disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 163 – 166°C

Purity (1) Chloride <*1.03*>—Dissolve 1.0 g of Felbinac in 40 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution by combining 0.30 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.011%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Felbinac according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Felbinac in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add acetone to make exactly 100 mL. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of heptane, acetone, and acetic acid (100) (50:25:1) to a distance of about 12 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Felbinac, previously dried, dissolve in 50 mL of methanol, add 15 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 21.22 mg of $C_{14}H_{12}O_2$

Containers and storage Containers—Tight containers.

Felbinac Cataplasm

フェルビナクパップ

Felbinac Cataplasm contains not less than 90.0% and not more than 110.0% of the labeled amount of felbinac ($C_{14}H_{12}O_2$: 212.24).

Method of preparation Prepare as directed under Cataplasms/Gel Patches, with Felbinac.

Identification Weigh a quantity of Felbinac Cataplasm, equivalent to 10 mg of Felbinac, cut into minute pieces, add 20 mL of methanol, shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of felbinac for assay in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, acetone and acetic acid (100) (50:25:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the principal spot obtained from the sample solution and the spot obtained from the standard solution is the same.

pH Being specified separately when the drug is granted approval based on the Law.

Adhesiveness Being specified separately when the drug is granted approval based on the Law.

Drug release Being specified separately when the drug is granted approval based on the Law.

Assay Take exactly a quantity of Felbinac Cataplasm, equivalent to 70 mg of felbinac ($C_{14}H_{12}O_2$), cut into minute pieces, add 150 mL of methanol, and heat under a reflux condenser. After cooling, separate the extraction liquid, add 20 mL of water to the residue, heat in a water bath at 75°C for 10 minutes, then add 150 mL of methanol, and heat under a reflux condenser. After cooling, separate the extraction liquid, add 150 mL of methanol to the residue, and heat under a reflux condenser. After cooling, separate the extraction liquid, wash the residue and vessels with a small amount of methanol, combine the extraction liquids and washings, and add methanol to make exactly 500 mL. Pipet 6 mL of this solution, add exactly 3 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 35 mg of felbinac for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 250 mL. Pipet 6 mL of this solution, add exactly 3 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of felbinac to that of the internal standard.

$$\text{Amount (mg) of felbinac } (C_{14}H_{12}O_2) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of felbinac for assay taken

Internal standard solution—A solution of indometacin in methanol (1 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 mn).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 1.5 mL of phosphoric acid add 300 mL of water, then dissolve 5 g of sodium lauryl sulfate, and add water to make 500 mL. To this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of felbinac is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, felbinac and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of felbinac to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Felbinac Tape

フェルビナクテープ

Felbinac Tape contains not less than 90.0% and not more than 110.0% of the labeled amount of felbinac ($C_{14}H_{12}O_2$: 212.24).

Method of preparation Prepare as directed under Tapes/Plasters, with Felbinac.

Identification Cut up a quantity of Felbinac Tape, equivalent to 5 mg of Felbinac, add 30 mL of ethanol (95), and heat under a reflux condenser. After cooling, separate the ethanol extract, add ethanol (95) to make 50 mL, and filter. To 5 mL of the filtrate add ethanol (95) to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 251 nm and 255 nm.

Adhesiveness Being specified separately when the drug is granted approval based on the Law.

Drug release Being specified separately when the drug is granted approval based on the Law.

Assay Take exactly a quantity of Felbinac Tape, equivalent to 35 mg of felbinac ($C_{14}H_{12}O_2$), cut up them, add 60 mL of acetone, treat with ultrasonic waves, and heat under a reflux condenser. After cooling, separate the acetone extract, and repeat the extraction twice more with 60 mL each of acetone by heating under a reflux condenser. After cooling, separate the extract, wash the residue and vessel with a small volume of acetone, combine the washings and the extracts, and add acetone to make exactly 250 mL. Pipet 6 mL of this solution, add exactly 2 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 14 mg of felbinac for assay, previously dried at 105°C for 3 hours, and dissolve in acetone to make exactly 100 mL. Pipet 6 mL of this solution, add exactly 2 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of felbinac to that of the internal standard.

$$\text{Amount (mg) of felbinac } (C_{14}H_{12}O_2) = M_S \times Q_T/Q_S \times 5/2$$

M_S: Amount (mg) of felbinac for assay taken

Internal standard solution—A solution of indomethacin in acetone (1 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (500:500:1).

Flow rate: Adjust so that the retention time of felbinac is about 7 minutes.

System suitability—

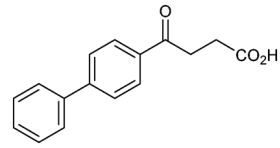
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, felbinac and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of felbinac to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fenbufen

フェンブフェン



$C_{16}H_{14}O_3$: 254.28
4-(Biphenyl-4-yl)-4-oxobutanoic acid
[*36330-85-5*]

Fenbufen, when dried, contains not less than 98.0% of fenbufen ($C_{16}H_{14}O_3$).

Description Fenbufen occurs as a white crystalline powder. It has a bitter taste.

It is sparingly soluble in acetone, slightly soluble in metha-

nol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point: about 188°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Fenbufen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fenbufen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Take 2.0 g of Fenbufen, add 2 mL of sulfuric acid, and carbonize by gentle heating, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Fenbufen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.1 g of Fenbufen in 20 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (80:20:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

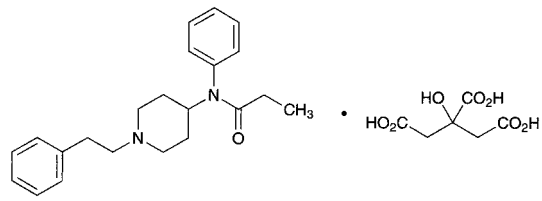
Assay Weigh accurately about 0.2 g of Fenbufen, previously dried, dissolve in 100 mL of ethanol (99.5), and titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 25.43 mg of $C_{16}H_{14}O_3$

Containers and storage Containers—Tight containers.

Fentanyl Citrate

フェンタニルクエン酸塩



$C_{22}H_{28}N_2O.C_6H_8O_7$: 528.59
N-(1-Phenethylpiperidin-4-yl)-*N*-phenylpropanamide monocitrate
[*990-73-8*]

Fentanyl Citrate contains not less than 98.0% of fentanyl citrate ($C_{22}H_{28}N_2O.C_6H_8O_7$), calculated on the dried basis.

Description Fentanyl Citrate occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in water and in ethanol (95), and very slightly soluble in diethyl ether.

Identification **(1)** Dissolve 0.05 g of Fentanyl Citrate in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fentanyl Citrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Fentanyl Citrate (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for citrate.

pH <*2.54*> Dissolve 0.10 g of Fentanyl Citrate in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

Melting point <*2.60*> 150 – 154°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of Fentanyl Citrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Fentanyl Citrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.2 g, in vacuum, silica gel, 60°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.5 g).

Assay Weigh accurately about 75 mg of Fentanyl Citrate, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 10.57 mg of $C_{22}H_{28}N_2.C_6H_8O_7$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Ferrous Sulfate Hydrate

硫酸鉄水和物

$FeSO_4.7H_2O$: 278.01

Ferrous Sulfate Hydrate contains not less than 98.0% and not more than 104.0% of ferrous sulfate hydrate ($FeSO_4.7H_2O$).

Description Ferrous Sulfate Hydrate occurs as pale green, crystals or crystalline powder. It is odorless, and has an astringent taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is efflorescent in dry air, and its surface becomes yellowish brown in moist air.

Identification A solution of Ferrous Sulfate Hydrate (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for ferrous salt and for sulfate.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Ferrous Sulfate Hydrate in 20 mL of water and 1 mL of dilute sulfuric acid: the solution is clear.

(2) Acidity—To 5.0 g of powdered Ferrous Sulfate Hydrate add 50 mL of ethanol (95), shake well for 2 minutes, and filter the mixture. To 25 mL of the filtrate add 50 mL of water, 3 drops of bromothymol blue TS and 0.5 mL of dilute sodium hydroxide TS: a blue color develops.

(3) Heavy metals ⟨*1.07*⟩—Take 1.0 g of Ferrous Sulfate Hydrate in a porcelain dish, add 3 mL of aqua regia, and dissolve. Then evaporate on a water bath to dryness. To the residue add 5 mL of 6 mol/L hydrochloric acid TS, and dissolve. Transfer this solution to a separator. Wash the porcelain dish with two 5-mL portions of 6 mol/L hydrochloric acid TS, and combine the washings and the solution in the separator. Pour two 40-mL portions and one 20-mL portion of diethyl ether in the separator, shaking each time to mix. Allow to stand, and discard each separated diethyl ether layer. To the aqueous layer add 0.05 g of hydroxylammonium chloride, dissolve, and heat on a water bath for 10 minutes. Cool, adjust the solution to a pH of 3 to 4 by dropping ammonia solution (28), add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: take 2.5 mL of Standard Lead Solution in a porcelain dish, add 3 mL of aqua regia, and proceed as directed for the preparation of the test solution (not more than 25 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Ferrous Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

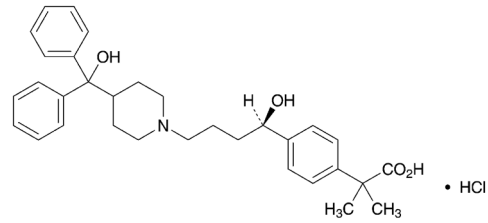
Assay Dissolve about 0.7 g of Ferrous Sulfate Hydrate, accurately weighed, in a mixture of 20 mL of water and 20 mL of dilute sulfuric acid, add 2 mL of phosphoric acid, and immediately titrate ⟨*2.50*⟩ with 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS
= 27.80 mg of $FeSO_4.7H_2O$

Containers and storage Containers—Tight containers.

Fexofenadine Hydrochloride

フェキソフェナジン塩酸塩



and enantiomer

$C_{32}H_{39}NO_4.HCl$: 538.12

2-(4-{(1*RS*)-1-Hydroxy-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butyl}phenyl)-2-methylpropanoic acid monohydrochloride [*153439-40-8*]

Fexofenadine Hydrochloride contains not less than 98.0% and not more than 102.0% of fexofenadine hydrochloride ($C_{32}H_{39}NO_4.HCl$), calculated on the anhydrous basis.

Description Fexofenadine Hydrochloride occurs as a white crystalline powder.

It is very soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

A solution of Fexofenadine Hydrochloride in methanol (3 in 100) shows no optical rotation.

Fexofenadine Hydrochloride shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Fexofenadine Hydrochloride in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fexofenadine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fexofenadine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Fexofenadine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Fexofenadine Hydrochloride in a mixture of water and methanol (1:1) (3 in 200) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Fexofenadine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 7.51 g of sodium dihydrogen phosphate dihydrate and 0.84 g of sodium

perchlorate in 1000 mL of water, and adjust to pH 2.0 with phosphoric acid. In a mixture of this solution and acetonitrile for liquid chromatography (1:1) dissolve 25 mg of Fexofenadine Hydrochloride to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than fexofenadine obtained from the sample solution is not larger than the peak area of fexofenadine obtained from the standard solution. For the areas of the peaks, having the relative retention time of about 1.8 and about 3.3 to fexofenadine, multiply their relative response factor, 1.5 and 0.9, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of fexofenadine, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fexofenadine are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fexofenadine is not more than 2.0%.

Water <2.48> Not more than 0.5% (0.25 g, coulometric titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve 7.51 g of sodium dihydrogen phosphate dihydrate and 0.84 g of sodium perchlorate in 1000 mL of water, and adjust to pH 2.0 with phosphoric acid. In a mixture of this solution and acetonitrile for liquid chromatography (1:1) dissolve accurately weighed about 25 mg each of Fexofenadine Hydrochloride and Fexofenadine Hydrochloride RS (separately determine the water <2.48> in the same manner as Fexofenadine Hydrochloride), to make exactly 25 mL each. Pipet 3 mL each of these solutions, add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of fexofenadine in each solution.

$$\text{Amount (mg) of fexofenadine hydrochloride } (C_{32}H_{39}NO_4.HCl) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Fexofenadine Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 650 mL of a solution, prepared by dissolving 7.51 g of sodium dihydrogen phosphate dihydrate and 0.84 g of sodium perchlorate in 1000 mL of water and adjusting to pH 2.0 with phosphoric acid, add 350 mL of acetonitrile for liquid chromatography and 3 mL of triethylamine.

Flow rate: Adjust so that the retention time of fexofenadine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fexofenadine are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fexofenadine is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fexofenadine Hydrochloride Tablets

フェキソフェナジン塩酸塩錠

Fexofenadine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4.HCl$: 538.12).

Method of preparation Prepare as directed under Tablets, with Fexofenadine Hydrochloride.

Identification To an amount of powdered Fexofenadine Hydrochloride Tablets, equivalent to 40 mg of Fexofenadine Hydrochloride, add 100 mL of methanol, and shake well. Filter, discard the first 10 mL of the filtrate, and determine the absorption spectrum of the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 257 nm and 261 nm.

Uniformity of dosage units <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Fexofenadine Hydrochloride Tablets add $V/5$ mL of diluted acetic acid (100) (17 in 10,000), shake until the tablet is disintegrated. Add $3V/5$ mL of acetonitrile for liquid chromatography, shake well, add a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly V mL so that each mL contains about 0.3 mg of fexofenadine hydrochloride ($C_{32}H_{39}NO_4.HCl$). Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Fexofenadine Hydrochloride RS (separately determine the water <2.48> in the same manner as Fexofenadine Hydrochloride), and dissolve in a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly 200 mL. Pipet

6 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl)
$= M_S \times A_T/A_S \times 3V/500$

M_S: Amount (mg) of Fexofenadine Hydrochloride RS taken, calculated on the anhydrous basis

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Fexofenadine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Fexofenadine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 30 μg of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Fexofenadine Hydrate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Fexofenadine Hydrochloride), dissolve in 5 mL of methanol, add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of fexofenadine in each solution.

Dissolution rate (%) with respect to the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Fexofenadine Hydrochloride RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.1 g of sodium dihydrogen phosphate dihydrate, 0.3 mL of phosphoric acid and 0.5 g of sodium perchlorate in 300 mL of water, add 700 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of fexofenadine is about 3.5 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fexofenadine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fexofenadine is not more than 2.0%.

Assay To 20 Fexofenadine Hydrochloride Tablets add $V/5$ mL of diluted acetic acid (100) (17 in 10,000), and shake until the tablets are disintegrated. Then, add $3V/5$ mL of acetonitrile for liquid chromatography, shake well, and add a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly V mL so that each mL contains about 1.2 mg of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl). Pipet 15 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 45 mg of Fexofenadine Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Fexofenadine Hydrochloride), and dissolve in a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly 200 mL. Pipet 20 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of fexofenadine in each solution.

Amount (mg) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl) in 1 tablet
$= M_S \times A_T/A_S \times V/750$

M_S: Amount (mg) of Fexofenadine Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 1000 mL of diluted acetic acid (100) (17 in 10,000) add 15 mL of a mixture of triethylamine and acetonitrile for liquid chromatography (1:1), and adjust to pH 5.25 with phosphoric acid. To 16 volumes of this solution add 9 volumes of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of fexofenadine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fexofenadine are not less than 7000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fexofenadine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Filgrastim (Genetical Recombination)

フィルグラスチム(遺伝子組換え)

MTPLGPASSL PQSFLLKCLE QVRKIQGDGA ALQEKLCATY KLCHPEELVL
LGHSLGIPWA PLSSCPSQAL QLAGCLSQLH SGLFLYQGLL QALEGISPEL
GPTLDTLQLD VADFATTIWQ QMEELGMAPA LQPTQGAMPA FASAFQRRAG
GVLVASHLQS FLEVSYRVLR HLAQP

$C_{845}H_{1339}N_{223}O_{243}S_9$: 18798.61
[*121181-53-1*]

Filgrastim (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant *N*-methionyl human granulocyte colony-stimulating factor consisting of 175 amino acid residues. It has a stimulating effect on neutrophil production.

It contains not less than 0.45 mg and not more than 0.55 mg of protein per mL, and not less than 1.0×10^8 units per mg of protein.

Description Filgrastim (Genetical Recombination) occurs as a clear and colorless liquid.

Identification (1) Take a volume of Filgrastim (Genetical Recombination), equivalent to 5 to 10 μg of protein depending on the size of polyacrylamide gel for filgrastim, and add 10 μL of water. To 3 volumes of this solution add 1 volume of buffer solution for filgrastim sample, and use this solution as the sample solution. Separately, take a volume of Filgrastim RS which contains equal amount of protein to Filgrastim (Genetical Recombination) used above, proceed as directed for the sample solution, and use the solution so obtained as the standard solution. Set a polyacrylamide gel for filgrastim up to the electrophoresis apparatus, and put a necessary amount of buffer solution for SDS-polyacrylamide gel electrophoresis in the upper and lower reservoirs. Pipet the all amount of the sample solution and standard solution into each well of the gel, and start the electrophoresis setting the electrode of the lower reservoir as the anode. Stop the electrophoresis when the bromophenol blue band has been migrated to about the lower end of the gel. When stain the gel with a staining solution, which is prepared by dissolving 1.25 g of Coomassie brilliant blue R250 in a mixture of 450 mL of methanol, 100 mL of acetic acid (100) and water to make 1000 mL, stained bands obtained from the sample solution appear as similar migrating image at the same position as those obtained from the standard solution.

(2) Take a volume of Filgrastim (Genetical Recombination) and Filgrastim RS, equivalent to about 80 μg of protein, add 200 μL of the buffer solution for enzyme digestion, and add water to make 390 μL. To each of these solution add 10 μL of a solution containing 50 μg of V8 protease in 250 μL of water, incubate at 25°C for 17 to 19 hours, then add 18 μL of a mixture of water and trifluoroacetic acid (19:1) to stop the reaction, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 70 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. When the chromatograms obtained from these solutions are compared, both chromatograms show the similar peaks at the same retention time.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 2.1 mm in inside diameter and 25 cm in length, packed with butylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of water and trifluoroacetic acid (1000:1).

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (9000:1000:9).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	98	2
2 – 30	98 → 70	2 → 30
30 – 85	70 → 50	30 → 50
85 – 90	50 → 2	50 → 98
90 – 100	2	98

Flow rate: 0.20 mL per minute.

System suitability—

System performance: When the procedure is run with 70 μL of the standard solution under the above operating conditions, the resolutions between each adjacent peakpair of the major 8 peaks, which are eluted after the solvent peak appeared within 10 minutes, are not less than 1.5.

pH <*2.54*> 3.7 – 4.3

Purity (1) Multimers—Perform the test with 250 μL of Filgrastim (Genetical Recombination) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate their amounts of the peaks by the area percentage method; the total amount of the peaks other than filgrastim is not more than 2%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.8 g of sodium chloride in 10 mL of dilute acetic acid and 900 mL of water, adjust to pH 5.5 with sodium hydroxide TS, then add 250 mg of sodium lauryl sulfate, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of filgrastim is about 17 minutes.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the time when the elution of filgrastim is completed.

System suitability—

Test for required detectability: Measure exactly 10 μL of Filgrastim (Genetical Recombination), and add the mobile phase to make exactly 1000 μL. Confirm that the peak area of filgrastim obtained with 250 μL of this solution is 0.7 to 1.3% of that obtained with 250 μL of Filgrastim (Genetical Recombination).

System performance: When the procedure is run with 10

μL of a solution containing 12.5 mg of egg albumin and 12.5 mg of myoglobin in 5 mL of water under the above operating conditions, egg albumin and myoglobin are eluted in this order with the resolution between these peaks being not less than 1.7.

System repeatability: When the test is repeated 6 times with 250 μL of Filgrastim (Genetical Recombination) under the above operating conditions, the relative standard deviation of the peak area of filgrastim is not more than 2.5%.

(2) Charge isomer—Perform the test with 100 μL of Filgrastim (Genetical Recombination) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the each peak area by the automatic integration method. Calculate their amounts of the peaks by the area percentage method; the amount of charge isomer, having the relative retention time of about 0.87 to filgrastim, is not more than 3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 35 mm in length, packed with strongly acidic ion-exchange non-porous resin for liquid chromatography (2.5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: To 900 mL of water add 1.14 mL of acetic acid (100), adjust to pH 5.4 with sodium hydroxide TS, and add water to make 1000 mL.

Mobile phase B: Dissolve 5.84 g of sodium chloride in 1.14 mL of acetic acid (100) and 900 mL of water, adjust to pH 5.4 with sodium hydroxide TS, and add water to make 1000 mL.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	100	0
2 – 10	100 → 40	0 → 60
10 – 11	40 → 100	60 → 0
11 – 20	100	0

Flow rate: Adjust so that the retention time of filgrastim is about 14 minutes.

Time span of measurement: From 6 minutes to 17 minutes.

System suitability—

Test for required detectability: Confirm that when perform the test with 100 μL of the system suitability test solution for filgrastim under the above operating conditions, the content of charge isomer is between 1.4 to 2.6%.

System performance: When the procedure is run with 100 μL of the system suitability test solution for filgrastim under the above operating conditions, charge isomer peak and filgrastim are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 100 μL of Filgrastim (Genetical Recombination) under the above operating conditions, the relative standard deviation of the peak area of filgrastim is not more than 2.5%.

(3) Host cell proteins Being specified separately when the drug is granted approval based on the Law.

(4) DNA Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins <*4.01*> Less than 0.25 EU/mL.

Assay (1) Protein content—Perform the test with exactly 200 μL each of Filgrastim (Genetical Recombination) and Filgrastim RS as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of filgrastim.

Amount (mg) of protein in 1 mL of Filgrastim (Genetical Recombination) $= C \times A_T/A_S$

C: Protein concentration (mg/mL) of Filgrastim RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, 1-propanol and trifluoroacetic acid (699:300:1).

Mobile phase B: A mixture of 1-propanole, water and trifluoroacetic acid (800:199:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	90	10
2 – 13	90 → 70	10 → 30
13 – 15	70 → 0	30 → 100
15 – 18	0	100

Flow rate: Adjust so that the retention time of filgrastim is about 15 minutes.

System suitability—

System performance: When the procedure is run with 200 μL of a solution prepared by dissolving 1 mg of uracil and 2 mg of diphenyl in 100 mL of a mixture of water, 1-propanol and trifluoroacetic acid (649:350:1) under the above operating conditions, uracil and diphenyl are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 200 μL of Filgrastim RS under the above operating conditions, the relative standard deviation of the peak area of filgrastim is not more than 2.5%.

(2) Specific activity—

(i) Test cell: 32D clone3 cell.

(ii) Sample dilution solution for assay: To Iscove's modified Dulbecco's fluid medium for filgrastim add 200 mmol/L L-glutamine solution and fetal calf serum to make 1 vol% and 5 vol% solution, respectively, and sterilize by filtration.

(iii) Standard solutions Dilute Filgrastim RS by the sample dilution solution for assay to prepare not less than 5 serial dilutions started from any concentration S_H so that all of their protein concentrations are within the range of 0.5 to 6 ng/mL, and use them as the standard solutions.

(iv) Sample solutions Dilute Filgrastim (Genetical Recombination) by the sample dilution solution for assay to prepare not less than 5 serial dilutions in equal ratio started from any concentration U_H so that all of their protein concentrations are within the range of 0.5 to 6 ng/mL, and use them as the sample solutions.

(v) Procedure The procedure before stopping the incubation should be performed under aseptic condition.

Transfer exactly 100 μL of each concentration of the standard solutions and sample solutions to the wells of 96-well flat bottom microplates. Not less than three plates are prepared for both standard solutions and sample solutions. Add exactly 100 μL of a test cell suspension containing 1×10^5 cells per mL in the sample dilution solution for assay to each well, and incubate under atmosphere of 5% carbon dioxide at 37 ± 2°C for 21 to 27 hours. After incubation, add 40 μL of fluorogenic substrate TS to each well, incubate under the same conditions as above for 21 to 51 hours, and measure fluorescence intensities at excitation wavelength 530 to 560 nm and at measurement wavelength 590 nm, using fluorescence microplate reader. Use the data from at least 3 plates and not less than 3 concentrations of the standard solution and sample solution for the calculation.

(vi) Calculation Transform each concentration of the sample solutions and standard solutions to common logarithm, and name them as x_U and x_S, respectively, and their totals are named as X_U and X_S, respectively. The fluorescence intensities obtained from the sample solution and the standard solution are named as y_U and y_S, and their totals are named as Y_U and Y_S, respectively. The numbers of the concentrations of the sample solution and the standard solution are named as n_U and n_S, respectively, the number of the plate is r. Calculate the specific activity of Filgrastim (Genetical Recombination) by the following equation, using the protein content (mg/mL) obtained in (1).

Specific activity (unit/mg) of Filgrastim (Genetical Recombination)

$$= \text{antilog } M \times \text{biological activity of Filgrastim RS (unit/mL)} \times \frac{\text{dilution factor for } U_H}{\text{dilution factor for } S_H} \times \frac{U_H}{S_H} \times \frac{1}{\text{protein content (mg/mL) obtained in the Assay (1)}}$$

$$M = X_S/n_S - X_U/n_U - (\Sigma Y_S/n_S r - \Sigma Y_U/n_U r)/b$$

$$b = (Sxy_S + Sxy_U)/(Sxx_S + Sxx_U)$$

$$Sxy_S = \Sigma x_S Y_S - X_S \Sigma Y_S/n_S$$

$$Sxy_U = \Sigma x_U Y_U - X_U \Sigma Y_U/n_U$$

$$Sxx_S = r\Sigma x_S^2 - rX_S^2/n_S$$

$$Sxx_U = r\Sigma x_U^2 - rX_U^2/n_U$$

The necessary requirements for validity of the test are following three items:

1) F's is not less than F_1 against $m = n_S(r-1)$ shown in the table below, and F'u is not less than F_1 against $m = n_U(r-1)$ shown in the table.

$$F'_S = V_{RS}/V_{ES}$$

$$V_{RS} = Sxy_S^2/Sxx_S$$

$$V_{ES} = \{\Sigma y_S^2 - \Sigma(Y_S^2/r)\}/\{n_S(r-1)\}$$

$$F'_U = V_{RU}/V_{EU}$$

$$V_{RU} = Sxy_U^2/Sxx_U$$

$$V_{EU} = \{\Sigma y_U^2 - \Sigma(Y_U^2/r)\}/\{n_U(r-1)\}$$

2) F' is smaller than F_1 against $m = (n_S + n_U)(r-1)$ shown in the table below.

$$F' = V_P/V_E$$

$$V_P = Sxy_S^2/Sxx_S + Sxy_U^2/Sxx_U - (Sxy_S + Sxy_U)^2/(Sxx_S + Sxx_U)$$

$$V_E = \{\Sigma y_S^2 + \Sigma y_U^2 - \Sigma(Y_S^2/r) - \Sigma(Y_U^2/r)\}/\{(n_S + n_U)(r-1)\}$$

3) $L \leqq 0.3$

$$L = 2/b(1-g)\sqrt{V_E F_1\{(1-g)(1/n_S r + 1/n_U r) + (\Sigma Y_S/n_S r - \Sigma Y_U/n_U r)^2/b^2(Sxx_S + Sxx_U)\}}$$

F_1: Value against $m = (n_S + n_U)(r-1)$ shown in the table.

$$g = V_E F_1/b^2(Sxx_S + Sxx_U)$$

Value of F_1 against m

m	F_1	m	F_1	m	F_1
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Hermetic containers. Storage—Not exceeding 10°C, avoiding freezing.

Filgrastim (Genetical Recombination) Injection

フィルグラスチム(遺伝子組換え)注射液

Filgrastim (Genetical Recombination) Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of filgrastim (genetical recombination) ($C_{845}H_{1339}N_{223}O_{243}S_9$: 18798.61).

Method of preparation Prepare as directed under Injections, with Filgrastim (Genetical Recombination).

Description Filgrastim (Genetical Recombination) Injection is a clear and colorless liquid.

Identification Take a volume of Filgrastim (Genetical Recombination) Injection, equivalent to 5 to 10 μg of Filgrastim (Genetical Recombination) depending on the size of polyacrylamide gel for filgrastim, and add 0 to 16 μL of water. To 3 volumes of this solution add 1 volume of buffer solution for filgrastim sample so that each mL contains about 0.19 mg of protein, and use this solution as the sample solution. Then, proceed as directed in the Identification (1) under Filgrastim (Genetical Recombination).

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Multimers—Proceed as directed in the Purity (1) under Filgrastim (Genetical Recombination) using a volume of Filgrastim (Genetical Recombination) Injection, equivalent to about 125 μg of Filgrastim (Genetical Recombination). Where, the test for required detectability and the system repeatability under the system suitability are tested using Filgrastim RS.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.25 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Biological activity Calculate the biological activity in 1 ampoule or syringe of Filgrastim (Genetical Recombination) Injection by the following equation, using the biological actively in 1 mL of Filgrastim (Genetical Recombination) Injection determined as directed in the Assay (2) under Filgrastim (Genetical Recombination) and the labeled volume of Filgrastim (Genetical Recombination) Injection: it is not less than 70% and not more than 140% of the target biological activity (unit).

Biological activity (unit) in 1 ampoule or syringe of Filgrastim (Genetical Recombination) Injection
= antilog M × biological activity (unit/mL) of Filgrastim RS × dilution factor for U_H/dilution factor for S_H × U_H/S_H × labeled volume (mL) of Filgrastim (Genetical Recombination) Injection

where, the target biological activity (unit) is calculated by the following formula.

Target biological activity (unit)
= 1.5×10^8 (unit/mg) × labeled amount (mg) of Filgrastim (Genetical Recombination) in labeled volume (mL)

Assay Perform the test with an exact volume each of Filgrastim (Genetical Recombination) Injection and Filgrastim RS, equivalent to about 100 μg of Filgrastim (Genetical Recombination), as directed in the Assay (1) under Filgrastim (Genetical Recombination).

Calculate the amount of filgrastim in 1 mL of Filgrastim (Genetical Recombination) Injection by following formula.

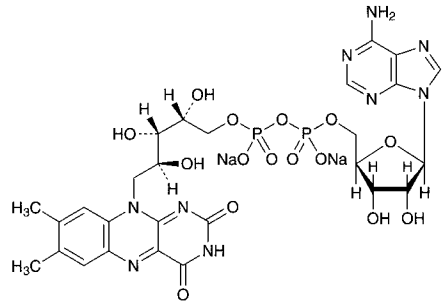
$$\text{Amount (mg) of filgrastim in 1 mL} = C \times A_T/A_S \times V_S/V_T$$

C: Protein concentration (mg/mL) of Filgrastim RS
V_S: Amount (μL) of Filgrastim RS taken
V_T: Amount (μL) of Filgrastim (Genetical Recombination) Injection taken

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, not exceeding 10°C avoiding freezing.

Flavin Adenine Dinucleotide Sodium

フラビンアデニンジヌクレオチドナトリウム



$C_{27}H_{31}N_9Na_2O_{15}P_2$: 829.51
Disodium adenosine 5′-[(2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypentyl diphosphate]
[*84366-81-4*]

Flavin Adenine Dinucleotide Sodium contains not less than 93.0% of flavin adenine dinucleotide sodium ($C_{27}H_{31}N_9Na_2O_{15}P_2$), calculated on the anhydrous basis.

Description Flavin Adenine Dinucleotide Sodium occurs as an orange-yellow to light yellow-brown powder. It is odorless or has a slight, characteristic odor, and has a slightly bitter taste.

It is freely soluble in water, and practically insoluble, in methanol, in ethanol (95), in ethyleneglycol and in diethyl ether.

It is hygroscopic.

It is decomposed by light.

Identification **(1)** A solution of Flavin Adenine Dinucleotide Sodium (1 in 100,000) is light yellow-green in color, and shows a strong yellow-green fluorescence. To 5 mL of the solution add 0.02 g of hydrosulfite sodium: the color and the fluorescence of the solution disappear, and gradually reappear when the solution is shaken in air. Add dilute hydrochloric acid or sodium hydroxide TS dropwise: the fluorescence of the solution disappears.

(2) Determine the infrared absorption spectrum of Flavin Adenine Dinucleotide Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Flavin Adenine Dinucleotide Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. To the residue add 10 mL of diluted nitric acid (1 in 50), boil for 5 minutes, and after cooling, neutralize with ammonia TS, then filter the solution if necessary: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt and the Qualitative Tests ⟨*1.09*⟩ (1) and (3) for phosphate.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -21.0 – $-25.5°$ (0.3 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Flavin Adenine Dinucleotide Sodium in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g

of Flavin Adenine Dinucleotide Sodium in 10 mL of water: the solution is clear and orange-yellow in color.

(2) Free phosphoric acid—Weigh accurately about 0.02 g of Flavin Adenine Dinucleotide Sodium, dissolve in 10 mL of water, and use this solution as the sample solution. Separately, measure exactly 2 mL of Standard Phosphoric Acid Solution, add 10 mL of water, and use this solution as the standard solution. To each of the sample solution and standard solution add 2 mL of diluted perchloric acid (100 in 117), then add 1 mL of hexaammonium heptamolybdate TS and 2 mL of 2,4-diaminophenol dihydrochloride TS, respectively, shake, add water to make exactly 25 mL, and allow to stand at 20±1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared in the same manner with 2 mL of water, as the blank, and determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 730 nm, respectively: the amount of free phosphoric acid is less than 0.25%.

$$\text{Amount (\%) of free phosphoric acid } (H_3PO_4) = 1/M \times A_T/A_S \times 5.16$$

M: Amount (mg) of flavin adenine dinucleotide sodium taken, calculated on the anhydrous basis

(3) Heavy metals <1.07>—Proceed with 1.0 g of Flavin Adenine Dinucleotide Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 2.0 g of Flavin Adenine Dinucleotide Sodium according to Method 3, and perform the test (not more than 1 ppm).

(5) Related substances—Dissolve 0.10 g of Flavin Adenine Dinucleotide Sodium in 200 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area, A, of flavin adenine dinucleotide and the total area, S, of peaks other than flavin adenine dinucleotide by the automatic integration method: $S/(A + S)$ is not more than 0.10.

Operating conditions—

Column, column temperature, mobile phase, flow rate, and time span of measurement: Proceed as directed in the operating conditions in the Procedure (ii) under the Assay (1).

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Procedure (ii) under the Assay (1).

Test for required detectability: To exactly 2 mL of the sample solution add the mobile phase to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of flavin adenine dinucleotide obtained from 20 μL of the solution for system suitability test is equivalent to 8 to 12% of that obtained from 20 μL of the sample solution.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0%.

Water <2.48> Take 50 mL of a mixture of methanol for water determination and ethyleneglycol for water determination (1:1) into a dry titration flask, and titrate with Karl Fischer TS for water determination until end point. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, transfer quickly to the titration flask, add an excess and constant volume of Karl Fischer TS for water determination, dissolve by stirring for 10 minutes, and perform the test: the water content is not more than 10.0%.

Assay (1) Procedure (i) Total flavin content—Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, and dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution, add 5 mL of zinc chloride TS, and heat in a water bath for 30 minutes. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Riboflavin RS, previously dried at 105°C for 2 hours, dissolve in 200 mL of diluted acetic acid (100) (1 in 100) by warming, cool, add water to make exactly 500 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 450 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\text{Total amout (mg) of flavin} = M_S \times A_T/A_S \times 4/5$$

M_S: Amount (mg) of Riboflavin RS taken

(ii) Peak area ratio of flavin adenine dinucleotide—Dissolve 0.1 g of Flavin Adenine Dinucleotide Sodium in 200 mL of water, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography <2.01> according to the following conditions. Determine the peak area, A of flavin adenine dinucleotide, and the total area, S, of the peaks other than flavin adenine dinucleotide by the automatic integration method.

$$\text{Peak area ratio of flavin adenine dinucleotide} = 1.08A/(1.08A + S)$$

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 450 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (1 in 500) and methanol (4:1).

Flow rate: Adjust so that the retention time of flavin adenine dinucleotide is about 10 minutes.

Time span of measurement: About 4.5 times as long as the retention time of flavin adenine dinucleotide.

System suitability—

Test for required detectability: To exactly 2 mL of the sample solution add water to make exactly 20 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add water to make exactly 20 mL. Confirm that the peak area of flavin adenine dinucleotide obtained from 5 μL of this solution is equivalent to 8 to 12% of that obtained from 5 μL of the solution for system suitability test.

System performance: Dissolve 20 mg each of Flavin Adenine Dinucleotide Sodium and riboflavin sodium phosphate in 100 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, flavin adenine dinucleotide and riboflavin phosphate are eluted in

this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0%.

(2) Calculation

Amount (mg) of flavin adenine dinucleotide sodium ($C_{27}H_{31}N_9Na_2O_{15}P_2$)
$= f_T \times f_R \times 2.2040$

f_T: Total amount (mg) of flavin in Flavin Adenine Dinucleotide Sodium obtained from the procedure (i)

f_R: Peak area ratio of flavin adenine dinucleotide in Flavin Adenine Dinucleotide Sodium obtained from the procedure (ii)

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Flavoxate Hydrochloride

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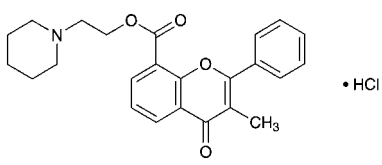

$C_{24}H_{25}NO_4.HCl$: 427.92
2-(Piperidin-1-yl)ethyl 3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxylate monohydrochloride
[*3717-88-2*]

Flavoxate Hydrochloride, when dried, contains not less than 99.0% of flavoxate hydrochloride ($C_{24}H_{25}NO_4.HCl$).

Description Flavoxate Hydrochloride occurs as white, crystals or crystalline powder.

It is sparingly soluble in acetic acid (100) and in chloroform, slightly soluble in water and in ethanol (95), and practically insoluble in acetonitrile and in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Flavoxate Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flavoxate Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Flavoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Flavoxate Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Flavoxate Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 80 mg of Flavoxate Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 20 mL, then pipet 1 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, reduced pressure, silica gel, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Flavoxate Hydrochloride, previously dried, add 10 mL of acetic acid (100) and 40 mL of acetonitrile to dissolve, add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
$= 42.79$ mg of $C_{24}H_{25}NO_4.HCl$

Containers and storage Containers—Tight containers.

Flecainide Acetate

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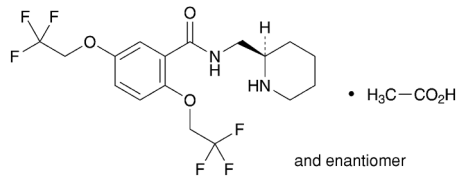

$C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$: 474.39
N-[(2*RS*)-Piperidin-2-ylmethyl]-2,5-bis(2,2,2-trifluoroethoxy)benzamide monoacetate
[*54143-56-5*]

Flecainide Acetate, when dried, contains not less than 98.0% and not more than 101.0% of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$).

Description Flecainide Acetate occurs as a white crystalline powder, having slightly a characteristic or acetic acid like odor.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), and sparingly soluble in water.

A solution of Flecainide Acetate in methanol (1 in 25) shows no optical rotation.

Melting point: about 150°C (with decomposition).

Identification **(1)** Dissolve 20 mg of Flecainide Acetate in 1 mL of water, add 1 mL of a solution of acetaldehyde (1 in 20), and shake. To this solution add dropwise at the same time 1 – 2 drops each of sodium pentacyanonitrosylferrate (III) dihydrate solution (1 in 10) and sodium hydrogen carbonate TS: a blue precipitate is formed.

(2) Determine the absorption spectrum of a solution of

Flecainide Acetate in ethanol (95) (13 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Flecainide Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Flecainide Acetate responds to the Qualitative Tests ⟨1.09⟩ (1) for acetate.

pH ⟨2.54⟩ The pH of a solution of 0.5 g of Flecainide Acetate in 20 mL of water is 6.7 to 7.1.

Purity **(1)** Clarity and color of solution—Dissolve 0.25 g of Flecainide Acetate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨1.07⟩—Transfer 1.0 g of Flecainide Acetate in a porcelain crucible, and heat gentry to carbonize. After cooling, add 2 mL of sulfuric acid, heat carefully until white fumes are no longer evolved, then proceed according to Method 2 to prepare the test solution, and perform the test. Prepare the control solution as follows: Place 2 mL each of sulfuric acid and hydrochloric acid in a porcelain crucible, evaporate on a water bath, then evaporate to dryness on a sand bath, add to the residue 3 drops of hydrochloric acid, then proceed in the same manner as for the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) 2-Aminomethylpiperidine—Dissolve exactly 0.25 g of Flecainide Acetate in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve exactly 50 mg of 2-aminomethylpiperidine in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and ammonia solution (28) (20:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in methanol (1 in 500), and heat at 105°C for 2 to 5 minutes: the spot obtained from the sample solution, corresponding to the spot obtained from the standard solution, is not more intense than the spot from the standard solution.

(4) Related substances—Dissolve 0.25 g of Flecainide Acetate in 25 mL of a mixture of water and acetonitrile (71:29), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (71:29) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (71:29) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak other than flecainide obtained from the sample solution is not larger than the peak area of flecainide obtained from the standard solution, and the total area of the peaks other than flecainide from the sample solution is not larger than 2.5 times the peak area of flecainide from the standard solution. For the areas of the peaks, having the relative retention time of about 1.5 and about 2.9 to flecainide, multiply their relative response factors, 0.3 and 1.7, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile, acetic acid (100) and tetrabutylammonium hydroxide-methanol TS (142:58:2:1), adjusted to pH 5.8 with ammonia solution (28).

Flow rate: Adjust so that the retention time of flecainide is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of flecainide, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of water and acetonitrile (71:29) to make exactly 10 mL. Confirm that the peak area of flecainide obtained from 20 μL of this solution is equivalent to 7 – 13% of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of flecainide are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flecainide is not more than 2.0%.

Loss on drying ⟨2.41⟩ Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 2 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.6 g of Flecainide Acetate, previously dried, dissolve in 100 mL of acetic acid (100), and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.44 mg of $C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Flecainide Acetate Tablets

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Flecainide Acetate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$: 474.39).

Method of preparation Prepare as directed under Tablets, with Flecainide Acetate.

Identification To an amount of powdered Flecainide Acetate Tablets, equivalent to 0.2 g of Flecainide Acetate, add 4 mL of methanol, shake for 20 minutes, then centrifuge and use the supernatant liquid as the sample solution. Separately, dissolve 0.1 g of flecainide acetate in 2 mL of methanol, and use this solution as the standard solution. Perform the test

with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone and ammonia solution (28) (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from standard solution show the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Completely disintegrate 1 tablet of Flecainide Acetate Tablets in $4V/5$ mL of a solution of lactic acid (1 in 500) with the aid of ultrasonic waves. After allowing to stand for 30 minutes while swirling occasionally, add a solution of lactic acid (1 in 500) to make exactly V mL so that each mL contains about 1 mg of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$), and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add a solution of lactic acid (1 in 500) to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$) $= M_S \times A_T/A_S \times V/25$

M_S: Amount (mg) of flecainide acetate for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Flecainide Acetate Tablets is not less than 70%.

Start the test with 1 tablet of Flecainide Acetate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of flecainide acetate for assay, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 2 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 296 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$) $= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of flecainide acetate for assay taken
C: Labeled amount (mg) of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$) in 1 tablet

Assay Accurately weigh the mass of not less than 20 Flecainide Acetate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$), add 80 mL of a solution of lactic acid (1 in 500), agitate for 5 minutes with the aid of ultrasonic waves, then add a solution of lactic acid (1 in 500) to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add a solution of lactic acid (1 in 500) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of flecainide acetate for assay, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 2 hours, dissolve in a solution of lactic acid (1 in 500) to make exactly 50 mL. Pipet 10 mL of this solution, add a solution of lactic acid (1 in 500) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 296 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

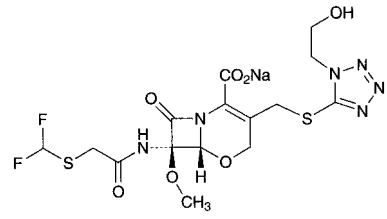
Amount (mg) of flecainide acetate ($C_{17}H_{20}F_6N_2O_3.C_2H_4O_2$) $= M_S \times A_T/A_S \times 4$

M_S: Amount (mg) of flecainide acetate for assay taken

Containers and storage Containers—Tight containers.

Flomoxef Sodium

フロモキセフナトリウム



$C_{15}H_{17}F_2N_6NaO_7S_2$: 518.45
Monosodium (6*R*,7*R*)-7-{[(difluoromethylsulfanyl)acetyl]amino}-3-[1-(2-hydroxyethyl)-1*H*-tetrazol-5-ylsulfanylmethyl]-7-methoxy-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*92823-03-5*]

Flomoxef Sodium contains not less than 870 μg (potency) and not more than 985 μg (potency) per mg, calculated on the anhydrous basis. The potency of Flomoxef Sodium is expressed as mass (potency) of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$: 496.47).

Description Flomoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (99.5).

Identification **(1)** Decompose 0.01 g of Flomoxef Sodium as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. To 2 mL of the test solution so obtained add 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1): blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Flomoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Flomoxef Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the ^1H spectrum of a solution of Flomoxef

Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 3.5 ppm, a single signal or a sharp multiple signal B at around δ 3.7 ppm, and a single signal C at around δ 5.2 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:1.

(5) Flomoxef Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: -8 – $-13°$ (1 g calculated on the anhydrous basis, a mixture of water and ethanol (99.5) (4:1), 50 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.5 g of Flomoxef Sodium in 5 mL of water is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Flomoxef Sodium in 10 mL of water: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS and 12 mL of Iron (III) Chloride CS add 35 mL of diluted dilute hydrochloric acid (1 in 10). To 5.0 mL of this solution add 5.0 mL of diluted dilute hydrochloric acid (1:10).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Flomoxef Sodium in a quartz crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—To 1.0 g of Flomoxef Sodium 5 mL of sulfuric acid and 5 mL of nitric acid, heat carefully until the solution changes to colorless to light yellow with occasional addition of 2 mL of nitric acid. After cooling, add 10 mL of ammonium oxalate TS, heat until white fumes evolve, and concentrate to 2 to 3 mL. After cooling, add water to make 10 mL, and perform the test using this solution as the test solution: the color is not darker than that of the control solution.

Control solution: Proceed to prepare a solution in the same manner as the test solution without Flomoxef Sodium, and transfer 10 mL of the solution so obtained to the generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed in the same manner as the test solution (not more than 2 ppm).

(4) 1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol—Use the sample solution obtained in the Assay as the sample solutions. Weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard: the amount of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol is not more than 1.0% of the amount of Flomoxef Sodium calculated on the anhydrous basis.

Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol ($C_3H_6N_4OS$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol taken

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol obtained with 5 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 3 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard is not more than 1.0%.

Water <2.48> Not more than 1.5% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Flomoxef Sodium and Flomoxef Triethylammonium RS, equivalent to about 50 mg (potency), and dissolve each in exactly 50 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of flomoxef to that of the internal standard.

Amount [μg (potency)] of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Flomoxef Triethylammonium RS taken

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 246 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 – 10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra-*n*-butylammonium bromide in water to make 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust so that the retention time of flomoxef is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, flomoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 3 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of flomoxef to that of the internal standard is

not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Not exceeding 5°C.

Flomoxef Sodium for Injection

注射用フロモキセフナトリウム

Flomoxef Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$: 496.47).

Method of preparation Prepare as directed under Injections, with Flomoxef Sodium.

Description Flomoxef Sodium for Injection occurs as white to light yellowish white, friable masses or powder.

Identification Proceed as directed in the Identification (3) under Flomoxef Sodium.

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving an amount of Flomoxef Sodium for Injection, equivalent to 0.5 g (potency) of Flomoxef Sodium, in 5 mL of water is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Flomoxef Sodium for Injection, equivalent to 1.0 g (potency) of Flomoxef Sodium, in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) 1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol—Use the sample solution obtained in the Assay as the sample solution. Weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard. Calculate the amount of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol per 1 g (potency) of Flomoxef Sodium for Injection by the following formula: not more than 10 mg.

Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol ($C_3H_6N_4OS$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol taken

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Flomoxef Sodium.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol obtained from 5 μL of this solution is equivalent to 3.5 – 6.5% of that obtained from 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 3 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 1.5% (0.5 g, volumetric titration, back titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.025 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Flomoxef Sodium for Injection, and calculate the average mass of the content. Spread out thinly about 1 g of the content in a petri dish, allow the dish to stand in a desiccator containing a saturated solution of magnesium bromide without light exposure to equilibrate the sample to constant water content. Determine the water content, separately, with about 0.1 g of the sample according to the method described in Water. Weigh accurately an amount of the sample, equivalent to about 50 mg (potency) of Flomoxef Sodium, add exactly 50 mL of the internal standard solution to dissolve, add water to make 100 mL, and use this solution as the sample solution. Separately weigh accurately about 50 mg (potency) of Flomoxef Triethylammonium RS, add exactly 50 mL of the internal standard solution to dissolve, add water to make 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Flomoxef Sodium.

Amount [μg (potency)] of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$)
$= M_S \times Q_T/Q_S \times 1000$

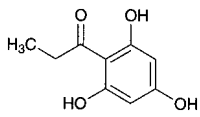
M_S: Amount [mg (potency)] of Flomoxef Triethylammonium RS taken

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injection may be used.

Flopropione

フロプロピオン



$C_9H_{10}O_4$: 182.17
1-(2,4,6-Trihydroxyphenyl)propan-1-one
[*2295-58-1*]

Flopropione contains not less than 98.0% and not more than 101.0% of flopropione ($C_9H_{10}O_4$), calculated on the anhydrous basis.

Description Flopropione occurs as a white to pale yellow-brown crystalline powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Flopropione in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flopropione as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 177 – 181°C

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of Flopropione according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Flopropione in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than flopropione obtained from the sample solution is not larger than 1/10 times the peak area of flopropione obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 267 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (114:86:1).

Flow rate: Adjust so that the retention time of flopropione is about 3 minutes.

Time span of measurement: About 7 times as long as the retention time of flopropione.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of flopropione obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 25 mg of ethyl parahydroxybenzoate in 30 mL of acetonitrile, and add the mobile phase to make 50 mL. To 2.5 mL of this solution add 2 mL of the sample solution and the mobile phase to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, flopropione and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flopropione is not more than 1.0%.

Water <*2.48*> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Flopropione, dissolve in 30 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 18.22 mg of $C_9H_{10}O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Flopropione Capsules

フロプロピオンカプセル

Flopropione Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of flopropione ($C_9H_{10}O_4$: 182.17).

Method of preparation Prepare as directed under the Capsules, with Flopropione.

Identification (1) Powder the contents of Flopropione Capsules. To a portion of the powder, equivalent to 60 mg of Flopropione, add 40 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of iron (III) nitrate TS: a red-purple color appears.

(2) Powder the contents of Flopropione Capsules. To a portion of the powder, equivalent to 90 mg of Flopropione, add 100 mL of ethanol (99.5), shake well, and filter. To 5 mL of the filtrate add ethanol (99.5) to make 50 mL. To 5 mL of this solution add ethanol (99.5) to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 283 nm and 287 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Flopropione Capsules add 43 mL of a mixture of water and phosphoric acid (86:1), and disintegrate the capsule in a water bath at 50°C. After cooling, add

a suitable amount of acetonitrile to make exactly V mL of a solution containing about 0.4 mg of flopropione ($C_9H_{10}O_4$) per mL. Stir the solution for 10 minutes, centrifuge a part of the solution at 3000 rpm for 5 minutes, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of flopropione } (C_9H_{10}O_4) = M_S \times A_T/A_S \times V/100$$

M_S: Amount (mg) of flopropione for assay taken, calculated on the anhydrous basis

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Flopropione Capsules is not less than 80%.

Start the test with 1 capsule of Flopropione Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 8.8 μg of flopropione ($C_9H_{10}O_4$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of flopropione for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Flopropione), and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 284 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using 0.1 mol/L hydrochloric acid TS as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of flopropione } (C_9H_{10}O_4) = M_S \times A_T/A_S \times V'/V \times 1/C \times 36$$

M_S: Amount (mg) of flopropione for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of flopropione ($C_9H_{10}O_4$) in 1 capsule

Assay Take out the contents of not less than 20 Flopropione Capsules, weigh accurately the mass of the contents, and power. Weigh accurately a part of the powder, equivalent to about 40 mg of flopropione ($C_9H_{10}O_4$), and add the mobile phase to make exactly 100 mL. Stir the solution for 10 minutes, centrifuge a part of this solution for 5 minutes at 3000 rpm, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of flopropione for assay (previously determine the water ⟨*2.48*⟩ in the same manner as Flopropione), add 70 mL of the mobile phase, and dissolve by exposure for 10 minutes to ultrasonic vibration. Add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of flopropione in each solution.

$$\text{Amount (mg) of flopropione } (C_9H_{10}O_4) = M_S \times A_T/A_S$$

M_S: Amount (mg) of flopropione for assay taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 267 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (114:86:1).

Flow rate: Adjust so that the retention time of flopropione is about 3 minutes.

System suitability—

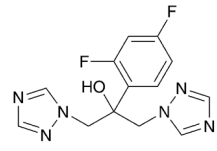
System performance: Dissolve 50 mg of flopropione in 50 mL of the mobile phase. To 20 mL of the solution add 25 mL of a solution prepared by dissolving 25 mg of ethyl parahydroxybenzoate in 30 mL of acetonitrile and add water to make 50 mL, and then add the mobile phase to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, Flopropione and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flopropione is not more than 1.0%.

Containers and storage Containers—Tight containers.

Fluconazole

フルコナゾール



$C_{13}H_{12}F_2N_6O$: 306.27

2-(2,4-Difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol [*86386-73-4*]

Fluconazole, when dried, contains not less than 99.0% and not more than 101.0% of fluconazole ($C_{13}H_{12}F_2N_6O$).

Description Fluconazole occurs as a white to pale yellowish white crystalline powder.

It is soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in dilute hydrochloric acid.

Identification **(1)** Dissolve 0.1 g of Fluconazole in 10 mL of dilute hydrochloric acid, and add 1 mL of Reinecke's salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Fluconazole in 0.01 mol/L hydrochloric acid-methanol TS (1 in 4000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluconazole as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨2.60⟩ 137 – 141°C

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.10 g of Fluconazole in 50 mL of water is clear and colorless.

(2) Heavy metals ⟨1.07⟩—Proceed with 1.0 g of Fluconazole according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 30 mg of Fluconazole in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the peak area of related substance I, having the relative retention time about 0.60 to fluconazole obtained from the sample solution is not larger than 6 times the peak area of fluconazole obtained from the standard solution, the area of the peak other than fluconazole and the related substance I from the sample solution is not larger than the peak area of fluconazole from the standard solution, and the total area of the peaks other than fluconazole from the sample solution is not larger than 8 times the peak area of fluconazole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of fluconazole is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of fluconazole, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of fluconazole obtained with 20 μL of this solution is equivalent to 35 to 65% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluconazole are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluconazole is not more than 2.0%.

Loss on drying ⟨2.41⟩ Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.25 g of Fluconazole, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.31 mg of $C_{13}H_{12}F_2N_6O$

Containers and storage Containers—Tight containers.

Fluconazole Capsules

フルコナゾールカプセル

Fluconazole Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$: 306.27).

Method of preparation Prepare as directed under Capsules, with Fluconazole.

Identification To an amount of powdered contents of Fluconazole Capsules, equivalent to 25 mg of Fluconazole, add 0.01 mol/L hydrochloric acid-methanol TS to make 100 mL, shake for 30 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩: it exhibits maxima between 259 nm and 263 nm and between 265 nm and 269 nm.

Uniformity of dosage units ⟨6.02⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To the total amount of the content of 1 capsule of Fluconazole Capsules add the mobile phase to make exactly 100 mL. Disperse the particles with the aid of ultrasonic waves, stir for 30 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 50 μg of fluconazole ($C_{13}H_{12}F_2N_6O$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of fluconazole ($C_{13}H_{12}F_2N_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/5$

M_S: Amount (mg) of fluconazole for assay taken

Dissolution ⟨6.10⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rates in 90 minutes of 50-mg capsule and 100-mg capsule are not less than 80% and not less than 70%, respectively.

Start the test with 1 capsule of Fluconazole Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 28 μg of fluconazole ($C_{13}H_{12}F_2N_6O$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of fluconazole for assay, previously dried at 105°C for 4 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of fluconazole in each solution.

Dissolution rate (%) with respect to the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of fluconazole for assay taken
C: Labeled amount (mg) of fluconazole ($C_{13}H_{12}F_2N_6O$) in 1 capsule

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluconazole are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluconazole is not more than 1.0%.

Assay Take out the contents from not less than 20 Fluconazole Capsules, weigh accurately, and powder, if necessary. Weigh accurately a quantity of the contents, equivalent to about 50 mg of fluconazole ($C_{13}H_{12}F_2N_6O$), and add the mobile phase to make exactly 100 mL. Disperse the particles with the aid of ultrasonic waves, stir for 30 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of fluconazole for assay, previously dried at 105°C for 4 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of fluconazole in each solution.

Amount (mg) of fluconazole ($C_{13}H_{12}F_2N_6O$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of fluconazole for assay taken

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 261 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: Dissolve 0.82 g of anhydrous sodium acetate in 1000 mL of water, and adjust to pH 5.0 with acetic acid (100). To 700 mL of this solution add 200 mL of methanol and 100 mL of acetonitrile.
Flow rate: Adjust so that the retention time of fluconazole is about 4 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluconazole are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluconazole is not more than 1.0%.

Containers and storage Containers—Tight containers.

Fluconazole Injection

フルコナゾール注射液

Fluconazole Injection is an aqueous injection.
It contains not less than 95.0% and not more than 105.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$: 306.27).

Method of preparation Prepare as directed under Injections, with Fluconazole.

Description Fluconazole Injection occurs as a clear and colorless liquid.

Identification **(1)** Take a volume of Fluconazole Injection, equivalent to 0.1 g of Fluconazole, and evaporate to dryness on a water bath. To the residue add 10 mL of dilute hydrochloric acid, shake, and filter. Add 1 mL of Reinecke salt TS to the filtrate: a light red precipitate is produced.
(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 259 nm and 263 nm, and between 264 nm and 268 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins <*4.01*> Less than 0.75 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Fluconazole Injection, equivalent to 10 mg of fluconazole ($C_{13}H_{12}F_2N_6O$), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of fluconazole for assay, previously dried at 105°C for 4 hours, dissolve in a solution of sodium chloride (9 in 1000) to make exactly 50 mL. Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 261 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

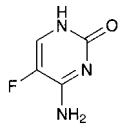
Amount (mg) of fluconazole ($C_{13}H_{12}F_2N_6O$)
$= M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of fluconazole for assay taken

Containers and storage Containers—Hermetic containers.

Flucytosine

フルシトシン



$C_4H_4FN_3O$: 129.09
5-Fluorocytosine
[*2022-85-7*]

Flucytosine, when dried, contains not less than 98.5% of flucytosine ($C_4H_4FN_3O$), and not less than 14.0% and not more than 15.5% of fluorine (F: 19.00).

Description Flucytosine occurs as a white crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in methanol, in ethanol (95), in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

It dissolves in 0.1 mol/L hydrochloric acid TS.

The pH of a solution of 1.0 g of Flucytosine in 100 mL of water is between 5.5 and 7.5.

It is slightly hygroscopic.

Melting point: about 295°C (with decomposition).

Identification **(1)** Add 0.2 mL of bromine TS to 5 mL of a solution of Flucytosine (1 in 500): a yellow-brown color of bromine TS is immediately discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is formed.

(2) Proceed with 0.1 g of Flucytosine as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. The solution responds to the Qualitative Tests <*1.09*> (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Flucytosine in 0.1 mol/L hydrochloric acid TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Flucytosine in 100 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 1.0 g of Flucytosine in 80 mL of water by heating on a water bath. After cooling, to 40 mL of this solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Fluoride—Dissolve 0.10 g of Flucytosine in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a 20-mL volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerrous nitrate TS (1:1:1), and add water to make 20 mL. Allow the mixture to stand for 1 hour, and use this solution as the sample solution. Separately, transfer 4.0 mL of Standard Fluorine Solution to a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerrous nitrate TS (1:1:1). Proceed in the same manner as directed in the preparation of the sample solution, and use this solution as the standard solution. Transfer 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) to a 20-mL volumetric flask, proceed in the same manner as directed in the preparation of the standard solution, and use this solution as the blank solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 600 nm, using the blank solution as the control as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: A_T is not larger than A_S (not more than 0.048%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Flucytosine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Flucytosine according to Method 2, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 50 mg of Flucytosine in 5 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Measure accurately 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 25 mL. Measure accurately 1 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (5:3:2) to a distance of about 12 cm, air-dry the plate, and observe the spots under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay **(1)** Flucytosine—Weigh accurately about 0.2 g of Flucytosine, previously dried, dissolve in 40 mL of acetic acid (100), add 100 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 12.91 mg of $C_4H_4FN_3O$

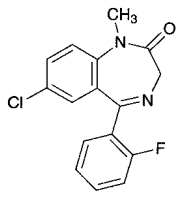
(2) Fluorine—Weigh accurately about 10 mg of Flucytosine, previously dried, and proceed as directed in the determination of fluorine under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide VS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fludiazepam

フルジアゼパム



$C_{16}H_{12}ClFN_2O$: 302.73
7-Chloro-5-(2-fluorophenyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*3900-31-0*]

Fludiazepam, when dried, contains not less than 99.0% of fludiazepam ($C_{16}H_{12}ClFN_2O$).

Description Fludiazepam occurs as white to light yellow, crystals or crystalline powder.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

Identification **(1)** Prepare the test solution with 0.01 g of Fludiazepam as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <*1.09*> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Fludiazepam in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Fludiazepam in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fludiazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Fludiazepam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 91 – 94°C

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Fludiazepam in 50 mL of diethyl ether, add 50 mL of water, and shake. Separate the water layer, wash it with two 20-mL portions of diethyl ether, and filter the water layer. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Fludiazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Fludiazepam in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethyl acetate (10:7) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Fludiazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.28 mg of $C_{16}H_{12}ClFN_2O$

Containers and storage Containers—Tight containers.

Fludrocortisone Acetate

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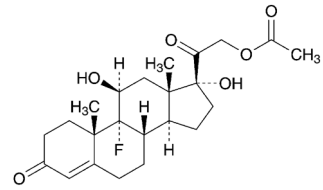


$C_{23}H_{31}FO_6$: 422.49
9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate
[*514-36-3*]

Fludrocortisone Acetate, when dried, contains not less than 97.5% and not more than 102.5% of fludrocortisone acetate ($C_{23}H_{31}FO_6$).

Description Fludrocortisone Acetate occurs as a white to pale yellow, crystals or crystalline powder.

It is soluble in acetone, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 220°C (with decomposition).

Identification **(1)** Prepare the test solution by proceeding with 10 mg of Fludrocortisone Acetate according to the Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide VS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <*1.09*> for fluoride.

(2) Determine the absorption spectrum of a solution of Fludrocortisone Acetate in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fludrocortisone Acetate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same

wavelengths.

(3) Determine the infrared absorption spectrum of Fludrocortisone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fludrocortisone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25}$: +131 – +138° (after drying, 0.1 g, acetone, 20 mL, 100 mm).

Purity **(1)** Heavy metals <1.07>—Proceed with 0.5 g of Fludrocortisone Acetate according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Fludrocortisone Acetate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than fludrocortisone acetate obtained from the sample solution is not larger than 1/4 times the peak area of fludrocortisone acetate obtained from the standard solution, and the total area of the peaks other than fludrocortisone acetate from the sample solution is not larger than 1/2 times the peak area of fludrocortisone acetate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and tetrahydrofuran (13:7).

Flow rate: Adjust so that the retention time of fludrocortisone acetate is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of fludrocortisone acetate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of fludrocortisone acetate obtained from 20 μL of this solution is equivalent to 4.0 to 6.0% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 2 mg each of Fludrocortisone Acetate and hydrocortisone acetate in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrocortisone acetate and fludrocortisone acetate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fludrocortisone acetate is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 100°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 25 mg each of Fludrocortisone Acetate and Fludrocortisone Acetate RS, previously dried, and dissolve separately in ethanol (95) to make exactly 100 mL. Pipet 4 mL each of these solutions, add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 238 nm.

Amount (mg) of fludrocortisone acetate ($C_{23}H_{31}FO_6$) = $M_S \times A_T/A_S$

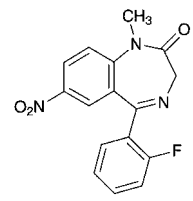
M_S: Amount (mg) of Fludrocortisone Acetate RS taken

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Flunitrazepam

フルニトラゼパム



$C_{16}H_{12}FN_3O_3$: 313.28

5-(2-Fluorophenyl)-1-methyl-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one

[*1622-62-4*]

Flunitrazepam, when dried, contains not less than 99.0% of flunitrazepam ($C_{16}H_{12}FN_3O_3$).

Description Flunitrazepam occurs as a white to pale yellow crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetic anhydride and in acetone, slightly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Flunitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flunitrazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 168 – 172°C

Purity **(1)** Chloride <1.03>—To 1.0 g of Flunitrazepam add 50 mL of water, allow to stand for 1 hour with occasional stirring, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Flunitrazepam according to Method 4 using a platinum cru-

cible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Flunitrazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, diethyl ether and ammonia solution (28) (200:100:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): number of the spots other than the principal spot from the sample solution is not more than 2, and they are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Flunitrazepam, previously dried, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.33 mg of $C_{16}H_{12}FN_3O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Fluocinolone Acetonide

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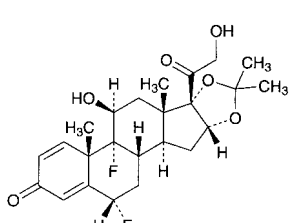


$C_{24}H_{30}F_2O_6$: 452.49
6α,9-Difluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione
[*67-73-2*]

Fluocinolone Acetonide, when dried, contains not less than 97.0% and not more than 102.0% of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$).

Description Fluocinolone Acetonide occurs as white, crystals or crystalline powder.

It is freely soluble in acetic acid (100) and in acetone, soluble in ethanol (99.5), sparingly soluble in methanol, and practically insoluble in water.

Melting point: 266 – 274°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** To 2 mg of Fluocinolone Acetonide add 2 mL of sulfuric acid: a yellow color is produced.

(2) Dissolve 0.01 g of Fluocinolone Acetonide in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Fluocinolone Acetonide as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <*1.09*> for fluoride.

(4) Determine the infrared absorption spectrum of Fluocinolone Acetonide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluocinolone Acetonide RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Fluocinolone Acetonide and Fluocinolone Acetonide RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +98 – +108° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 15 mg of Fluocinolone Acetonide in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than fluocinolone acetonide from the sample solution is not larger than the peak area of fluocinolone acetonide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water-saturated chloroform, methanol and acetic acid (100) (200:3:2).

Flow rate: Adjust so that the retention time of fluocinolone acetonide is about 12 minutes.

Time span of measurement: About 2 times as long as the retention time of fluocinolone acetonide, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of fluocinolone acetonide obtained from 20 μL of this solution is equivalent to 4 to 6% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 15 mg each of Fluocinolone Acetonide and triamcinolone acetonide in 25 mL of the mobile phase. To 5 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, triamcinolone acetonide and fluocinolone acetonide are eluted in this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of fluocinolone acetonide is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.2 g, in vacuum, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.2 g, platinum crucible).

Assay Weigh accurately about 20 mg each of Fluocinolone Acetonide and Fluocinolone Acetonide RS, previously dried, and dissolve in 40 mL each of methanol, add exactly 10 mL each of the internal standard solution, then add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fluocinolone acetonide to that of the internal standard.

Amount (mg) of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Fluocinolone Acetonide RS taken

Internal standard solution—A solution of ethyl parahydroxybenzoate (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust so that the retention time of fluocinolone acetonide is about 20 minutes.

System suitability—

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

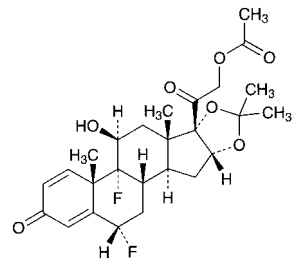
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluocinolone acetonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fluocinonide

フルオシノニド



$C_{26}H_{32}F_2O_7$: 494.52

6α,9-Difluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione 21-acetate

[*356-12-7*]

Fluocinonide, when dried, contains not less than 97.0% and not more than 103.0% of fluocinonide ($C_{26}H_{32}F_2O_7$).

Description Fluocinonide occurs as white, crystals or crystalline powder.

It is sparingly soluble in chloroform, slightly soluble in acetonitrile, in methanol, in ethanol (95) and in ethyl acetate, and practically insoluble in water.

It shows crystal polymorphism.

Identification **(1)** To 0.01 g of Fluocinonide add 4 mL of water and 1 mL of Fehling's TS, and heat: a red precipitate is formed.

(2) Prepare the test solution with 0.01 g of Fluocinonide as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ for fluoride.

(3) Determine the absorption spectrum of a solution of Fluocinonide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluocinonide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Fluocinonide and Fluocinonide RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare both spectra: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears in the absorption spectra, dissolve the sample and the RS in ethyl acetate, respectively, evaporate the ethyl acetate, and perform the test with the residue in the same manner.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +81 – +89° (after drying, 0.2 g, chloroform, 20 mL, 100 mm).

Purity Related substances—Dissolve 10 mg of Fluocinonide in 2 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (97:3)

to a distance of about 12 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g, platinum crucible).

Assay Weigh accurately about 20 mg each of Fluocinonide and Fluocinonide RS, previously dried, dissolve each in 50 mL of acetonitrile, to each add exactly 8 mL of the internal standard solution and water to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fluocinonide to that of the internal standard, respectively.

$$\text{Amount (mg) of fluocinonide } (C_{26}H_{32}F_2O_7) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Fluocinonide RS taken

Internal standard solution—A solution of propyl benzoate in acetonitrile (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of fluocinonide is about 8 minutes.

System suitability—

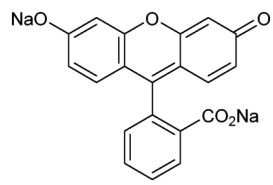
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fluocinonide and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluocinonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fluorescein Sodium

フルオレセインナトリウム



$C_{20}H_{10}Na_2O_5$: 376.27

Disodium 2-(6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate

[*518-47-8*]

Fluorescein Sodium contains not less than 98.5% of fluorescein sodium ($C_{20}H_{10}Na_2O_5$), calculated on the dried basis.

Description Fluorescein Sodium occurs as an orange powder. It is odorless, and tasteless.

It is freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** To a solution of Fluorescein Sodium (1 in 100) having a strong green fluorescence, add a large quantity of water: the fluorescence remains. Acidify the solution with hydrochloric acid: the fluorescence disappears. Then render the solution alkaline with sodium hydroxide TS: the fluorescence reappears.

(2) Place 1 drop of a solution of Fluorescein Sodium (1 in 2000) on a piece of filter paper: a yellow spot develops. Expose the spot, while moist, to the vapor of bromine for 1 minute and then to ammonia vapor: the yellow color of the spot changes to red.

(3) Char 0.5 g of Fluorescein Sodium by ignition, cool, mix the residue with 20 mL of water, and filter: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 1 g of Fluorescein Sodium in 10 mL of water: the solution is clear, and shows a red color.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.15 g of Fluorescein Sodium in 20 mL of water, add 6 mL of dilute nitric acid and water to make 30 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.355%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.20 g of Fluorescein Sodium in 30 mL of water, add 2.5 mL of dilute hydrochloric acid and water to make 40 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Zinc—Dissolve 0.10 g of Fluorescein Sodium in 10 mL of water, add 2 mL of hydrochloric acid, and filter. To the filtrate add 0.1 mL of potassium hexacyanoferrate (II) TS: no turbidity is produced immediately.

(5) Related substances—Dissolve 0.20 g of Fluorescein Sodium in exactly 10 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (30:15:1)

to a distance of about 10 cm, and air-dry the plate: any colored spot other than the principal spot does not appear.

Loss on drying ⟨*2.41*⟩ Not more than 10.0% (1 g, 105°C, constant mass).

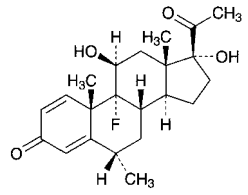
Assay Transfer about 0.5 g of Fluorescein Sodium, accurately weighed, to a separator. Dissolve in 20 mL of water, add 5 mL of dilute hydrochloric acid, and extract with four 20-mL portions of a mixture of 2-methyl-1-propanol and chloroform (1:1). Wash each extract successively with the same 10 mL of water. Evaporate the combined extracts on a water bath with the aid of a current of air. Dissolve the residue in 10 mL of ethanol (99.5), evaporate the solution on a water bath to dryness, dry the residue at 105°C for 1 hour, and weigh as fluorescein ($C_{20}H_{12}O_5$: 332.31).

Amount (mg) of fluorescein sodium ($C_{20}H_{10}Na_2O_5$)
= amount (mg) of fluorescein ($C_{20}H_{12}O_5$) × 1.132

Containers and storage Containers—Tight containers.

Fluorometholone

フルオロメトロン



$C_{22}H_{29}FO_4$: 376.46
9-Fluoro-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione
[*426-13-1*]

Fluorometholone, when dried, contains not less than 97.0% and not more than 103.0% of fluorometholone ($C_{22}H_{29}FO_4$).

Description Fluorometholone occurs as a white to light yellowish white, odorless, crystalline powder.

It is freely soluble in pyridine, slightly soluble in methanol, in ethanol (99.5) and in tetrahydrofuran, and practically insoluble in water and in diethyl ether.

Identification **(1)** Proceed with 7 mg of Fluorometholone as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the liquid responds to the Qualitative Tests ⟨*1.09*⟩ (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Fluorometholone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluorometholone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluorometholone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluorometholone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +52 – +60° (after drying, 0.1 g, pyridine, 10 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Fluorometholone according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Fluorometholone in 10 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add tetrahydrofuran to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography ⟨*2.03*⟩. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone and methanol (45:5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2 % (0.2 g, platinum crucible).

Assay Weigh accurately about 0.1 g each of Fluorometholone and Fluorometholone RS, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, and add diluted methanol (7 in 10) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and diluted methanol (7 in 10) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of fluorometholone to that of the internal standard.

Amount (mg) of fluorometholone ($C_{22}H_{29}FO_4$)
= $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Fluorometholone RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Diluted methanol (7 in 10).

Flow rate: Adjust so that the retention time of fluorometholone is about 8 minutes.

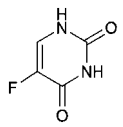
Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of fluorometholone and the internal standard in this order with the resolution between these peaks being not less than 4.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Fluorouracil

フルオロウラシル



$C_4H_3FN_2O_2$: 130.08
5-Fluorouracil
[*51-21-8*]

Fluorouracil, when dried, contains not less than 98.5% of fluorouracil ($C_4H_3FN_2O_2$), and not less than 13.1% and not more than 16.1% of fluorine (F: 19.00).

Description Fluorouracil occurs as white, crystals or crystalline powder. It is odorless.

It is freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 282°C (with decomposition).

Identification **(1)** Add 0.2 mL of bromine TS to 5 mL of a solution of Fluorouracil (1 in 500): the color of bromine TS is discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is formed.

(2) Proceed with 0.01 g of Fluorouracil as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ for fluoride.

(3) Determine the absorption spectrum of a solution of Fluorouracil in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity and color of solution—Add 20 mL of water to 0.20 g of Fluorouracil, and dissolve by warming: the solution is clear and colorless.

(2) Fluoride—Dissolve 0.10 g of Fluorouracil in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a 20-mL volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1), and add water to make 20 mL. Allow to stand for 1 hour, and use this solution as the sample solution. Separately, transfer 1.0 mL of Standard Fluorine Solution to a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1). Proceed in the same manner as directed for the preparation of the sample solution, and use this solution as the standard solution. Perform the test as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the sample solution at 600 nm is not larger than that of the standard solution (not more than 0.012%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Fluorouracil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic ⟨*1.11*⟩—To 1.0 g of Fluorouracil in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol to burn, and incinerate by strong heating at 750°C to 850°C. If a carbonized substance remains in this method, moisten with a small amount of nitric acid, and incinerate by strong heating. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve it by warming on a water bath, use this solution as the test solution, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Fluorouracil in 10 mL of water, and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (7:4:1) to a distance of about 12 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 80°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay **(1)** Fluorouracil—Weigh accurately about 0.2 g of Fluorouracil, previously dried, dissolve in 20 mL of *N*,*N*-dimethylformamide, and titrate ⟨*2.50*⟩ with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethylformamide TS). Perform a blank determination.

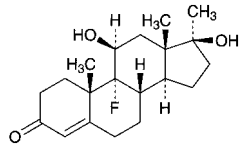
Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 13.01 mg of $C_4H_3FN_2O_2$

(2) Fluorine—Weigh accurately about 4 mg of Fluorouracil, previously dried, and proceed as directed in the determination of fluorine under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Fluoxymesterone

フルオキシメステロン



$C_{20}H_{29}FO_3$: 336.44
9-Fluoro-11β,17β-dihydroxy-17-methylandrost-4-en-3-one
[*76-43-7*]

Fluoxymesterone, when dried, contains not less than 97.0% and not more than 102.0% of fluoxymesterone ($C_{20}H_{29}FO_3$).

Description Fluoxymesterone occurs as white, crystals or

crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water.

It shows crystal polymorphism.

Identification **(1)** Dissolve 5 mg of Fluoxymesterone in 2 mL of sulfuric acid: a yellow color develops.

(2) Prepare the test solution with 0.01 g of Fluoxymesterone as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Fluoxymesterone in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluoxymesterone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Fluoxymesterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluoxymesterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Fluoxymesterone and Fluoxymesterone RS in ethanol (99.5), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +104 – +112° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 0.5 g of Fluoxymesterone according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.03 g of Fluoxymesterone in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, ethanol (95) and ethyl acetate (3:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.5 g, platinum crucible).

Assay Weigh accurately about 25 mg each of Fluoxymesterone and Fluoxymesterone RS, previously dried, dissolve each in the internal standard solution to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fluoxymesterone to that of the internal standard.

Amount (mg) of fluoxymesterone ($C_{20}H_{29}FO_3$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Fluoxymesterone RS taken

Internal standard solution—A solution of methylprednisolone in a mixture of chloroform and methanol (19:1) (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 1-chlorobutane, water-saturated 1-chlorobutane, tetrahydrofuran, methanol and acetic acid (100) (95:95:14:7:6).

Flow rate: Adjust so that the retention time of fluoxymesterone is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, fluoxymesterone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

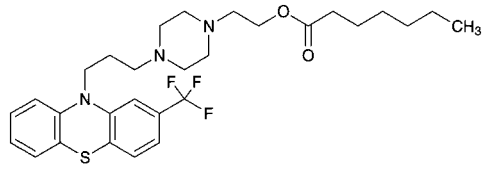
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluoxymesterone to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Fluphenazine Enanthate

フルフェナジンエナント酸エステル



$C_{29}H_{38}F_3N_3O_2S$: 549.69

2-(4-{3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl}piperazin-1-yl)ethyl heptanoate

[*2746-81-8*]

Fluphenazine Enanthate, when dried, contains not less than 98.5% of fluphenazine enanthate ($C_{29}H_{38}F_3N_3O_2S$).

Description Fluphenazine Enanthate is a light yellow to yellowish orange viscous liquid. It is generally clear, and can be opaque by producing crystals.

It is freely soluble in methanol and in diethyl ether, soluble in ethanol (95) and in acetic acid (100), and practically insoluble in water.

Identification **(1)** Prepare the test solution with 0.01 g of Fluphenazine Enanthate as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualita-

tive Tests <*1.09*> for fluoride.

(2) Dissolve 2 mg of Fluphenazine Enanthate in 200 mL of a solution of hydrochloric acid in methanol (17 in 2000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluphenazine Enanthate as directed in the liquid firm method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Fluphenazine Enanthate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.25 g of Fluphenazine Enanthate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, hexane and ammonia solution (28) (16:6:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Then spray evenly diluted sulfuric acid (1 in 2) on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

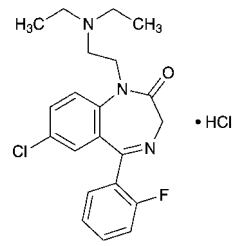
Assay Weigh accurately about 0.5 g of Fluphenazine Enanthate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.49 mg of $C_{29}H_{38}F_3N_3O_2S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Flurazepam Hydrochloride

フルラゼパム塩酸塩



$C_{21}H_{23}ClFN_3O.HCl$: 424.34
7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one monohydrochloride
[*36105-20-1*]

Flurazepam Hydrochloride, when dried, contains not less than 99.0% of flurazepam hydrochloride ($C_{21}H_{23}ClFN_3O.HCl$).

Description Flurazepam Hydrochloride occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, in ethanol (95), in ethanol (99.5) and in acetic acid (100).

Melting point: about 197°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Flurazepam Hydrochloride in sulfuric acid-ethanol TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flurazepam Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Flurazepam Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> Dissolve 1.0 g of Flurazepam Hydrochloride in 20 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Flurazepam Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Sulfate <*1.14*>—Perform the test with 1.5 g of Flurazepam Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Flurazepam Hydrochloride in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.05 g of Flurazepam Hydrochloride in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and

standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Place the plate in a chamber filled with ammonia vapor, allow to stand for about 15 minutes, and immediately develop the plate with a mixture of diethyl ether and diethylamine (39:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 3 spots other than the principal spot and the spot on the starting point from the sample solution appear, and are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

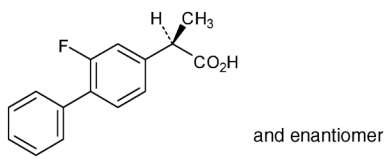
Assay Weigh accurately about 0.3 g of Flurazepam Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.22 mg of $C_{21}H_{23}ClFN_3O.HCl$

Containers and storage Containers—Tight containers.

Flurbiprofen

フルルビプロフェン



and enantiomer

$C_{15}H_{13}FO_2$: 244.26
(2*RS*)-2-(2-Fluorobiphenyl-4-yl)propanoic acid
[*5104-49-4*]

Flurbiprofen, when dried, contains not less than 98.0% of flurbiprofen ($C_{15}H_{13}FO_2$).

Description Flurbiprofen occurs as a white crystalline powder. It has a slightly irritating odor.

It is freely soluble in methanol, in ethanol (95), in acetone and in diethyl ether, soluble in acetonitrile, and practically insoluble in water.

A solution of Flurbiprofen in ethanol (95) (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Flurbiprofen in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flurbiprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 114 – 117°C

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 0.6 g of Flurbiprofen in 40 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015%).

(2) Heavy metals ⟨*1.07*⟩—Dissolve 2.0 g of Flurbiprofen in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Flurbiprofen in 10 mL of a mixture of water and acetonitrile (11:9), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (11:9) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than flurbiprofen from the sample solution is not larger than the peak area of flurbiprofen from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of flurbiprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (12:7:1).

Flow rate: Adjust so that the retention time of flurbiprofen is about 20 minutes.

Time span of measurement: About twice as long as the retention time of flurbiprofen, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of water and acetonitrile (11:9) to make exactly 25 mL. Confirm that the peak area of flurbiprofen obtained from 20 μL of this solution is equivalent to 16 to 24% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 0.04 g of flurbiprofen and 0.02 g of butyl parahydroxybenzoate in 100 mL of a mixture of water and acetonitrile (11:9). To 5 mL of this solution add a mixture of water and acetonitrile (11:9) to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and flurbiprofen are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flurbiprofen is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.10% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g, platinum crucible).

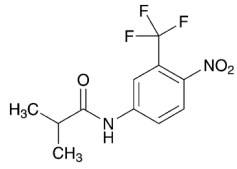
Assay Weigh accurately about 0.6 g of Flurbiprofen, previously dried, dissolve in 50 mL of ethanol (95), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.43 mg of $C_{15}H_{13}FO_2$

Containers and storage Containers—Well-closed containers.

Flutamide

フルタミド



$C_{11}H_{11}F_3N_2O_3$: 276.21
2-Methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide
[*13311-84-7*]

Flutamide, when dried, contains not less than 98.5% and not more than 101.5% of flutamide ($C_{11}H_{11}F_3N_2O_3$).

Description Flutamide occurs as a light yellow crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Flutamide in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Flutamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flutamide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Flutamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 109 – 113°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Flutamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 40 mg of Flutamide in 50 mL of methanol, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of them by the area percentage method: the amount of each peak other than flutamide is not more than 0.3%, and the total amount of the peaks other than flutamide is not more than 0.5%.

Operating conditions—

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Time span of measurement: About 2 times as long as the retention time of flutamide, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To 1 mL of the sample solution, add methanol to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add methanol to make exactly 20 mL. Confirm that the peak area of flutamide obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flutamide is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 40 mg each of Flutamide and Flutamide RS, previously dried, and dissolve separately in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of flutamide to that of the internal standard.

Amount (mg) of flutamide ($C_{11}H_{11}F_3N_2O_3$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Flutamide RS taken

Internal standard solution—A solution of testosterone in methanol (9 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.05 mol/L potassium dihydrogen phosphate TS (7:4).

Flow rate: Adjust so that the retention time of flutamide is about 12 minutes.

System suitability—

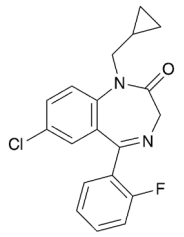
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, flutamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flutamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Flutoprazepam

フルトプラゼパム



$C_{19}H_{16}ClFN_2O$: 342.79
7-Chloro-1-cyclopropylmethyl-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*25967-29-7*]

Flutoprazepam, when dried, contains not less than 99.0% and not more than 101.0% of flutoprazepam ($C_{19}H_{16}ClFN_2O$).

Description Flutoprazepam occurs as a white to light yellow, crystals or crystalline powder.

It is freely soluble in ethyl acetate, soluble in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

Identification **(1)** Dissolve 2 mg of Flutoprazepam in 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flutoprazepam as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Flutoprazepam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 118 – 122°C

Purity **(1)** Chloride <*1.03*>—To 1.0 g of Flutoprazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Flutoprazepam according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Flutoprazepam in 20 mL of ethyl acetate, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethyl acetate to make exactly 50 mL. Pipet 1 mL of this solution, add ethyl acetate to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (3:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Loss on drying <*2.41*> Not more than 0.20% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Flutoprazepam, previously dried, dissolve in 70 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.28 mg of $C_{19}H_{16}ClFN_2O$

Containers and storage Containers—Well-closed containers.

Flutoprazepam Tablets

フルトプラゼパム錠

Flutoprazepam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of flutoprazepam ($C_{19}H_{16}ClFN_2O$: 342.79).

Method of preparation Prepare as directed under Tablets, with Flutoprazepam.

Identification To a quantity of powdered Flutoprazepam Tablets, equivalent to 10 mg of Flutoprazepam, add 20 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 100 mL. Centrifuge this solution, to 10 mL of the supernatant liquid add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 240 nm and 244 nm, between 279 nm and 285 nm, and between 369 nm and 375 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Flutoprazepam Tablets add 60 mL of the mobile phase, shake for 15 minutes to disintegrate, disperse the particle with the aid of ultrasonic waves, and add the mobile phase to make exactly V mL so that each mL contains about 20 μg of flutoprazepam ($C_{19}H_{16}ClFN_2O$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Proceed as directed in the Assay.

Amount (mg) of flutoprazepam ($C_{19}H_{16}ClFN_2O$)
$= M_S \times A_T/A_S \times V/1000$

M_S: Amount (mg) of flutoprazepam for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Flutoprazepam Tablets is not less than 70%.

Start the test with 1 tablet of Flutoprazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.2 μg of flutoprazepam ($C_{19}H_{16}ClFN_2O$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of flutoprazepam for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of flutoprazepam in each solution.

Dissolution rate (%) with respect to the labeled amount of flutoprazepam ($C_{19}H_{16}ClFN_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of flutoprazepam for assay taken
C: Labeled amount (mg) of flutoprazepam ($C_{19}H_{16}ClFN_2O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of flutoprazepam are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flutoprazepam is not more than 1.0%.

Assay Weigh accurately not less than 20 Flutoprazepam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2 mg of flutoprazepam ($C_{19}H_{16}ClFN_2O$), add 60 mL of the mobile phase, shake for 15 minutes, and add the mobile phase to make exactly 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of flutoprazepam for assay, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of flutoprazepam in each solution.

Amount (mg) of flutoprazepam ($C_{19}H_{16}ClFN_2O$)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of flutoprazepam for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and water (3:1).

Flow rate: Adjust so that the retention time of flutoprazepam is about 5 minutes.

System suitability—

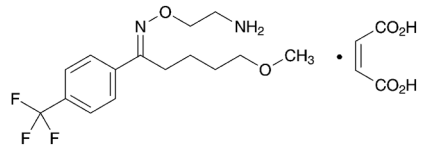
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of flutoprazepam are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flutoprazepam is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fluvoxamine Maleate

フルボキサミンマレイン酸塩



$C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$: 434.41
5-Methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one (*E*)-*O*-(2-aminoethyl)oxime monomaleate
[*61718-82-9*]

Fluvoxamine Maleate contains not less than 98.0% and not more than 101.0% of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$), calculated on the dried basis.

Description Fluvoxamine Maleate occurs as a white crystalline powder.

It is freely soluble in ethanol (99.5), and sparingly soluble in water.

Identification **(1)** Dissolve 10 mg of Fluvoxamine Maleate in 5 mL of water, neutralize with dilute sodium hydroxide TS, then add 1 mL of ninhydrin TS, and heat in a water bath at 60 – 70°C for 5 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Fluvoxamine Maleate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluvoxamine Maleate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluvoxamine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Fluvoxamine Maleate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Fluvoxamine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the TS disappears immediately.

Melting point <*2.60*> 120 – 124°C

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.5 g of Fluvoxamine Maleate in 50 mL

of water is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 1.0 g of Fluvoxamine Maleate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

(3) Sulfate <*1.14*>—Perform the test with 1.0 g of Fluvoxamine Maleate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Fluvoxamine Maleate according to Method 2, using alumina ceramic crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Related substances—Dissolve 20 mg of Fluvoxamine Maleate in 20 mL of a mixture of methanol for liquid chromatography and water (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention time of about 0.76, about 0.82, about 0.89, about 1.58 and about 1.66 to fluvoxamine, obtained from the sample solution are not larger than 1/5 times, 3/10 times, 7/10 times, 1/10 times and 1/10 times the peak area of fluvoxamine obtained from the standard solution, respectively, and the total area of the peaks other than fluvoxamine from the sample solution is not larger than 1.5 times the peak area of fluvoxamine from the standard solution. For the areas of the peaks, having the relative retention times of about 0.76, about 0.89, about 1.58 and about 1.66 to fluvoxamine, multiply their relative response factors, 0.87, 2.00, 0.67 and 2.76, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 12.67 g of diammonium hydrogen phosphate and 0.85 g of sodium 1-heptanesulfonate in 900 mL of water, adjust to pH 2.0 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of methanol for liquid chromatography.

Flow rate: Adjust so that the retention time of fluvoxamine is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of fluvoxamine, beginning after the peak of maleic acid.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 20 mL. Confirm that the peak area of fluvoxamine obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluvoxamine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluvoxamine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.1% (1 g, in vacuum, 50°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 20 mg each of Fluvoxamine Maleate and Fluvoxamine Maleate RS (separately determine the loss on drying <*2.41*> under the same condition as Fluvoxamine Maleate), dissolve each in 10 mL of the mobile phase, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fluvoxamine to that of the internal standard.

$$\text{Amount (mg) of fluvoxamine maleate } (C_{15}H_{21}F_3N_2O_2.C_4H_4O_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Fluvoxamine Maleate RS taken, calculated on the dried basis

Internal standard solution—A solution of diphenylamine in methanol (7 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.8 g of diammonium hydrogen phosphate and 0.8 g of sodium 1-heptanesulfonate in water to make 300 mL, add 700 mL of methanol, and adjust to pH 3.5 with phosphoric acid.

Flow rate: Adjust so that the retention time of fluvoxamine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fluvoxamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of fluvoxamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fluvoxamine Maleate Tablets

フルボキサミンマレイン酸塩錠

Fluvoxamine Maleate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$: 434.41).

Method of preparation Prepare as directed under Tablets, with Fluvoxamine Maleate.

Identification Powder Fluvoxamine Maleate Tablets. To a portion of the powder, equivalent to 0.1 g of Fluvoxamine Maleate, add 50 mL of water, shake, then allow to stand, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To 0.5 mL of the filtrate add 50 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 243 nm and 247 nm.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Fluvoxamine Maleate Tablets add 4 mL of water, disintegrate the tablet with the aid of ultrasonic waves, add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 50 mL, and filter. Pipet V mL of the filtrate, equivalent to about 6 mg of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$), add exactly 2 mL of the internal standard solution, then add a mixture of methanol for liquid chromatography and water (7:3) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times 6/V$

M_S: Amount (mg) of Fluvoxamine Maleate RS taken, calculated on the dried basis

Internal standard solution—A solution of diphenylamine in methanol for liquid chromatography (3 in 1000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Fluvoxamine Maleate Tablets is not less than 80%.

Start the test with 1 tablet of Fluvoxamine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μg of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Fluvoxamine Maleate RS (separately determine the loss on drying <*2.41*> under the same condition as Fluvoxamine Maleate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 245 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Fluvoxamine Maleate RS taken, calculated on the dried basis

C: Labeled amount (mg) of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$) in 1 tablet

Assay To 10 Fluvoxamine Maleate Tablets add 20 mL of water, disintegrate the tablets with the aid of ultrasonic waves, then add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 250 mL, and filter. Pipet V mL of the filtrate, equivalent to about 6 mg of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$), add exactly 2 mL of the internal standard solution, then add a mixture of methanol for liquid chromatography and water (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Fluvoxamine Maleate RS (separately determine the loss on drying <*2.41*> under the same condition as Fluvoxamine Maleate), and dissolve in a mixture of methanol for liquid chromatography and water (7:3) to make exactly 25 mL. Pipet 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add a mixture of methanol for liquid chromatography and water (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fluvoxamine to that of the internal standard.

Amount (mg) of fluvoxamine maleate ($C_{15}H_{21}F_3N_2O_2.C_4H_4O_4$) in 1 tablet
$= M_S \times Q_T/Q_S \times 3/V$

M_S: Amount (mg) of Fluvoxamine Maleate RS taken, calculated on the dried basis

Internal standard solution—A solution of diphenylamine in methanol for liquid chromatography (3 in 1000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Fluvoxamine Maleate.

System suitability—

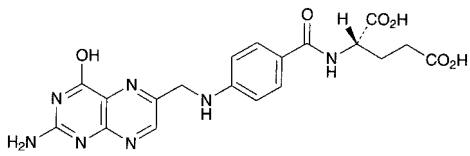
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fluvoxamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of fluvoxamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Folic Acid

葉酸



$C_{19}H_{19}N_7O_6$: 441.40
N-{4-[(2-Amino-4-hydroxypteridin-6-ylmethyl)amino]benzoyl}-L-glutamic acid
[*59-30-3*]

Folic Acid contains not less than 98.0% and not more than 102.0% of folic acid ($C_{19}H_{19}N_7O_6$), calculated on the anhydrous basis.

Description Folic Acid occurs as a yellow to orange-yellow crystalline powder. It is odorless.

It is practically insoluble in water, in methanol, in ethanol (95), in pyridine and in diethyl ether.

It dissolves in hydrochloric acid, in sulfuric acid, in dilute sodium hydroxide TS and in a solution of sodium carbonate decahydrate (1 in 100), and these solutions are yellow in color.

It is slowly affected by light.

Identification **(1)** Dissolve 1.5 mg of Folic Acid in dilute sodium hydroxide TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Folic Acid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) To 10 mL of the solution obtained in (1) add 1 drop of potassium permanganate TS, and mix well until the color changes to blue, and immediately observe under ultraviolet light (main wavelength: 365 nm): a blue fluorescence is produced.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Folic Acid in 10 mL of dilute sodium hydroxide TS : the solution is clear and yellow in color.

(2) Free amines—Pipet 30 mL of the sample solution obtained in the Assay, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of *p*-Aminobenzoyl Glutamic Acid RS, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in diluted ethanol (2 in 5) to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution, proceed as directed in the Assay, and perform the test as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Determine the absorbances, A_T and A_S, of subsequent solutions of the sample solution and standard solution at 550 nm: the content of free amines is not more than 1.0%.

$$\text{Content (\%) of free amines} = M_S/M_T \times A_T/A_S$$

M_T: Amount (mg) of Folic Acid taken, calculated on the anhydrous basis
M_S: Amount (mg) of *p*-Aminobenzoyl Glutamic Acid RS taken

Water <*2.48*> Not more than 8.5% (10 mg, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.5% (1 g).

Assay Weigh accurately about 50 mg each of Folic Acid and Folic Acid RS (separately, determine the water <*2.48*> in the same manner as Folic Acid). To each add 50 mL of dilute sodium hydroxide TS, mix well to dissolve, add dilute sodium hydroxide TS to make exactly 100 mL, and use these solutions as the sample solution and standard solution. To 30 mL each of these solutions, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 60 mL each of these solutions add 0.5 g of zinc powder, and allow to stand with frequent shaking for 20 minutes. Filter each mixture through a dry filter paper, and discard the first 10 mL of the filtrate. Pipet 10 mL each of the subsequent filtrate, and add water to make exactly 100 mL. To 4 mL each of solutions, accurately measured, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of a solution of sodium nitrite (1 in 1000), mix well, and allow to stand for 2 minutes. To each solution add 1 mL of a solution of ammonium amidosulfate (1 in 200), mix thoroughly, and allow to stand for 2 minutes. To each of these solutions, add 1 mL of a solution of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL. Separately, to 30 mL of the sample solution, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 18 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 4 mL of this solution, and prepare the blank solution in the same manner as the sample solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 4 mL of water in the same manner as a blank. Determine the absorbances, A_T, A_S and A_C, of the subsequent solution of the sample solution, the standard solution and the blank solution at 550 nm.

$$\text{Amount (mg) of folic acid } (C_{19}H_{19}N_7O_6) = M_S \times (A_T - A_C)/A_S$$

M_S: Amount (mg) of Folic Acid RS taken, calculated on the anyhdrous basis

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Folic Acid Injection

葉酸注射液

Folic Acid Injection is an aqueous injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$: 441.40).

Method of preparation Dissolve Folic Acid in water with the aid of Sodium Hydroxide or Sodium Carbonate, and prepare as directed under Injections.

Description Folic Acid Injection is a yellow to orange-yellow, clear liquid.

pH: 8.0 – 11.0

Identification **(1)** To a volume of Folic Acid Injection, equivalent to 1.5 mg of Folic Acid, add dilute sodium hydroxide TS to make 100 mL. Proceed as directed in the Iden-

tification (2) under Folic Acid, using this solution as the sample solution.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm and between 361 nm and 369 nm. Separately, determine the maximal absorbances of the sample solution, A_1 and A_2, between 255 nm and 257 nm and between 361 nm and 369 nm, respectively: the ratio of A_1/A_2 is between 2.80 and 3.00.

(3) Folic Acid Injection responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Folic Acid Injection, equivalent to about 50 mg of folic acid ($C_{19}H_{19}N_7O_6$) add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid RS (separately, determine the water <*2.48*> in the same manner as Folic Acid), dissolve in dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Proceed with exactly 30 mL each of the sample solution and standard solution as directed in the Assay under Folic Acid.

$$\text{Amount (mg) of folic acid } (C_{19}H_{19}N_7O_6) = M_S \times (A_T - A_C)/A_S$$

M_S: Amount (mg) of Folic Acid RS taken, calculated on the anhydrous basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Folic Acid Tablets

葉酸錠

Folic Acid Tablets contain not less than 90.0% and not more than 115.0% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$: 441.40).

Method of preparation Prepare as directed under Tablets, with Folic Acid.

Identification (1) Take a quantity of powdered Folic Acid Tablets, equivalent to 1.5 mg of Folic Acid, add 100 mL of dilute sodium hydroxide TS, shake, and filter. Discard the first 10 mL of the filtrate, use the subsequent filtrate as the sample solution, and proceed as directed in the Identification (2) under Folic Acid.

(2) Determine the absorption spectrum of the filtrate obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm and between 361 nm and 369 nm. Separately, determine the maximal absorbances of the filtrate, A_1 and A_2, between 255 nm and 257 nm and between 361 nm and 369 nm, respectively: the ratio of A_1/A_2 is between 2.80 and 3.00.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Folic Acid Tablets add 50 mL of dilute sodium hydroxide TS, shake frequently, and filter. Wash the residue with dilute sodium hydroxide TS, combine the filtrate and the washings, then add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 30 mL of the sample stock solution, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 60 mL of this solution, add 0.5 g of zinc powder, shake frequently, allow to stand for 20 minutes, and filter the solution through a dried filter paper. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 15 μg of folic acid ($C_{19}H_{19}N_7O_6$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid RS (separately determine the water <*2.48*> in the same manner as Folic Acid), and dissolve in dilute sodium hydroxide TS to make exactly 100 mL. Pipet 30 mL of this solutions, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 60 mL of this solution, add 0.5 g of zinc powder, shake frequently, allow to stand for 20 minutes, and filter the solution through a dried filter paper. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of sodium nitrite solution (1 in 1000) to them, mix, and allow to stand for 2 minutes. To these solutions add 1 mL of a solution of ammonium amidosulfate (1 in 200), shake, and allow them to stand for 2 minutes. To these solutions add 1 mL of a solution of *N*,*N*-diethyl-*N'*-1-naphthylethylenediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL. Separately, pipet 30 mL of the sample stock solution, add 20 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet V mL of this solution, and add water to make exactly V' mL so that each mL contains about 15 μg of folic acid ($C_{19}H_{19}N_7O_6$). With exactly 4 mL of this solution perform the same procedure described above for obtaining the sample solution, and use the solution so obtained as the blank solution. Determine the absorbances at 550 nm, A_T, A_S and A_C, of the solutions obtained from the sample solution and standard solution, and the blank solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a control solution obtained with 4 mL of water in the same manner as described above.

$$\text{Amount (mg) of folic acid } (C_{19}H_{19}N_7O_6) = M_S \times (A_T - A_C)/A_S \times V'/V \times 1/10$$

M_S: Amount (mg) of Folic Acid RS taken, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Folic Acid Tablets is not less than 75%.

Start the test with 1 tablet of Folic Acid Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of folic acid ($C_{19}H_{19}N_7O_6$), and use this solution as the

sample solution. Separately, weigh accurately about 20 mg of Folic Acid RS (separately determine the water <2.48> in the same manner as Folic Acid), and dissolve in the 2nd fluid for dissolution test to make exactly 100 mL. Pipet 2.5 mL of this solution, add the 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 280 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$

M_S: Amount (mg) of Folic Acid RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of folic acid ($C_{19}H_{19}N_7O_6$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Folic Acid Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of folic acid ($C_{19}H_{19}N_7O_6$). Add 50 mL of dilute sodium hydroxide TS, shake frequently, then filter into a 100-mL volumetric flask, and wash with dilute sodium hydroxide TS. To the combined filtrate and washings add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid RS (separately, determine the water <2.48> in the same manner as Folic Acid), dissolve in dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Proceed with exactly 30 mL each of the sample solution and standard solution as directed in the Assay under Folic Acid.

Amount (mg) of folic acid ($C_{19}H_{19}N_7O_6$)
$= M_S \times (A_T - A_C)/A_S$

M_S: Amount (mg) of Folic Acid RS taken, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Formalin

ホルマリン

Formalin contains not less than 35.0% and not more than 38.0% of formaldehyde (CH_2O: 30.03.)
It contains 5% to 13% of methanol to prevent polymerization.

Description Formalin is a clear, colorless liquid. Its vapor is irritating to the mucous membrane.
It is miscible with water and with ethanol (95).
When stored for a long time, especially in a cold place, it may become cloudy.

Identification **(1)** Dilute 2 mL of Formalin with 10 mL of water in a test tube, and add 1 mL of silver nitrate-ammonia TS: a gray precipitate is produced, or a silver mirror is formed on the wall of the test tube.
(2) To 5 mL of sulfuric acid in which 0.1 g of salicylic acid has been dissolved add 2 drops of Formalin, and warm the solution: a persistent, dark red color develops.

Purity Acidity—Dilute 20 mL of Formalin with 20 mL of water, and add 5.0 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of bromothymol blue TS: a blue color develops.

Residue on ignition <2.44> Not more than 0.06 w/v% (5 mL, after evaporation).

Assay Weigh accurately a weighing bottle containing 5 mL of water, add about 1 g of Formalin, and weigh accurately again. Add water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 50 mL of 0.05 mol/L iodine VS and 20 mL of potassium hydroxide TS, and allow to stand for 15 minutes at an ordinary temperature. To this mixture add 15 mL of dilute sulfuric acid, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH_2O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Formalin Water

ホルマリン水

Formalin Water contains not less than 0.9 w/v% and not more than 1.1 w/v% of formaldehyde (CH_2O: 30.03).

Method of preparation

Formalin	30 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare by mixing the above ingredients.

Description Formalin Water is a clear, colorless liquid. It has a slight odor of formaldehyde.
It is almost neutral.

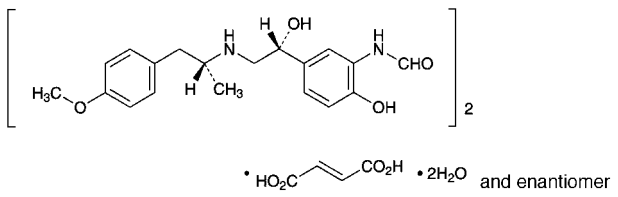
Assay Transfer 20 mL of Formalin Water, measured exactly, to a 100-mL volumetric flask containing 2.5 mL of 1 mol/L sodium hydroxide VS, and add water to make 100 mL. Pipet 10 mL of this solution, and proceed as directed in the Assay under Formalin.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH_2O

Containers and storage Containers—Tight containers.

Formoterol Fumarate Hydrate

ホルモテロールフマル酸塩水和物



$(C_{19}H_{24}N_2O_4)_2.C_4H_4O_4.2H_2O$: 840.91
N-(2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(1*RS*)-2-(4-methoxyphenyl)-1-methylethylamino]ethyl}phenyl)formamide hemifumarate monohydrate
[*43229-80-7*, anhydride]

Formoterol Fumarate Hydrate contains not less than 98.5% of formoterol fumarate [$(C_{19}H_{24}N_2O_4)_2.C_4H_4O_4$: 804.88], calculated on the anhydrous basis.

Description Formoterol Fumarate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Formoterol Fumarate Hydrate in methanol (1 in 100) shows no optical rotation.

Melting point: about 138°C (with decomposition).

Identification **(1)** Dissolve 0.5 g of Formoterol Fumarate Hydrate in 20 mL of 0.5 mol/L sulfuric acid TS, and extract with three 25-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10 mL of 0.5 mol/L sulfuric acid TS, and evaporate the ether layer under reduced pressure, and dry the residue at 105°C for 3 hours: the residue melts <*2.60*> at about 290°C (with decomposition, in a sealed tube).

(2) Determine the absorption spectrum of a solution of Formoterol Fumarate Hydrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Formoterol Fumarate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Formoterol Fumarate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related Substances—Dissolve 0.20 g of Formoterol Fumarate Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, 1,4-dioxane, ethanol (99.5) and ammonia solution (28) (20:20:10:3) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 4.0 – 5.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

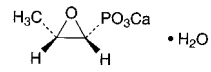
Assay Weigh accurately about 0.7 g of Formoterol Fumarate Hydrate, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 40.24 mg of $(C_{19}H_{24}N_2O_4)_2.C_4H_4O_4$

Containers and storage Containers—Tight containers.

Fosfomycin Calcium Hydrate

ホスホマイシンカルシウム水和物



$C_3H_5CaO_4P.H_2O$: 194.14
Monocalcium (2*R*,3*S*)-3-methyloxiran-2-ylphosphonate monohydrate
[*26016-98-8*]

Fosfomycin Calcium Hydrate is the calcium salt of a substance having antibacterial actively produced by the growth of *Streptomyces fradiae* or by the chemical synthesis.

It contains not less than 725 μg (potency) and not more than 805 μg (potency) per mg, calculated on the anhydrous basis. The potency of Fosfomycin Calcium Hydrate is expressed as mass (potency) of fosfomycin ($C_3H_7O_4P$: 138.06).

Description Fosfomycin Calcium Hydrate occurs as a white crystalline powder.

It is slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

Identification **(1)** Determine the infrared absorption spectrum of Fosfomycin Calcium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Fosfomycin Calcium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 300), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits a double signal at around δ 1.5 ppm, a duple double signal at around δ 2.9 ppm, a multiple signal at around δ 3.3 ppm, and no signal at around δ 1.4 ppm.

(3) A solution of Fosfomycin Calcium Hydrate (1 in 500) responds to the Qualitative Tests <*1.09*> (3) for calcium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −2.5 – −5.4° (0.5 g calculated on the anhydrous bases, 0.4 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (pH 8.5), 10 mL, 100 mm).

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Calcium Hydrate, add 40 mL of sodium periodate (107 in 10,000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 70 mg of potassium dihydrogen phosphate, proceed with this solution in the same manner as directed for the preparation of the sample stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the sample stock solution without using Fosfomycin Calcium Hydrate, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the sample stock solution, the standard stock solution, and the blank stock solution, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, mix, and add water to make exactly 25 mL, and use these solutions as the sample solution, the standard solution, and the blank solution, respectively. After allowing these solutions to stand at 20 ± 1°C for 30 minutes, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as a blank, and determine the absorbances at 740 nm, A_T, A_S and A_B, of the sample solution, the standard solution and the blank solution: the content of phosphorus is 15.2 – 16.7%.

$$\text{Amount (mg) of phosphorus (P)} = M_S \times (A_T - A_B)/(A_S - A_B) \times 0.228$$

M_S: Amount (mg) of potassium dihydrogen phosphate taken

Calcium Content Weigh accurately about 0.2 g of Fosfomycin Calcium Hydrate, add 4 mL of 1 mol/L Hydrochloric acid TS, and shake well until the sample is completely dissolved. To this solution add 100 mL of water, 9 mL of sodium hydroxide TS and 0.1 g of methylthymol blue-sodium chloride indicator, and titrate ⟨*2.50*⟩ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from clear blue to gray or gray-purple: calcium content is 19.6 – 21.7%. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.004 mg of Ca

Purity **(1)** Heavy metals ⟨*1.07*⟩—To 1.0 g of Fosfomycin Calcium Hydrate add 40 mL of 0.25 mol/L acetic acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Fosfomycin Calcium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

Water ⟨*2.48*⟩ Not more than 12.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Proteus* sp. (MB838)

(ii) Culture medium—Dissolve 5.0 g of peptone, 3.0 g of meat extract, 2.0 g of yeast extract, and 15 g of agar in 1000 mL of water, sterilize, and use as the agar media for base layer and seed layer with the pH of between 6.5 and 6.6 after sterilization.

(iii) Seeded agar layer—Incubate the test organism on the slant of the agar medium for transferring test organisms at 37°C for 40 – 48 hours. Subcultures at least 3 times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organisms in a Roux bottle, incubate at 37°C for 40 – 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water, and use this as the stock suspension of test organism. The amount of the water to be added is adjust so that the percent transmission at 560 nm of the suspension diluted ten times with water is 17%. Keep the stock suspension at 10°C or below and use within 7 days. Add 1.0 – 2.0 mL of the stock suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the deeded agar layer.

(iv) Standard solutions—Weigh accurately an amount of Fosfomycin Phenethylammonium RS equivalent to about 20 mg (potency), dissolve in 0.05 mol/L tris buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.05 mol/L tris buffer solution (pH 7.0) to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(v) Sample solutions—Weigh accurately an amount of Fosfomycin Calcium Hydrate equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L tris buffer solution (pH 7.0) to make exactly 50 mL. To exactly a suitable amount of this solution add 0.05 mol/L tris buffer solution (pH 7.0) to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Fosfomycin Calcium for Syrup

シロップ用ホスホマイシンカルシウム

Fosfomycin Calcium for Syrup is a preparation for syrups which is suspended before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of fosfomycin ($C_3H_7O_4P$: 138.06).

Method of preparation Prepare as directed under Syrups, with Fosfomycin Calcium Hydrate.

Identification **(1)** To an amount of Fosfomycin Calcium for Syrup, equivalent to 40 mg (potency) of Fosfomycin Calcium Hydrate, add 10 mL of warm water, shake for 10 to 20 minutes, and collect the insoluble substances by filtration. Dissolve the substances in 3 mL of a solution of perchloric acid (1 in 4), add 1 mL of 0.1 mol/L sodium periodate solution, and warm in a water bath at 60°C for 30 minutes. After cooling, add 50 mL of water, neutralize the solution with a saturated solution of sodium hydrogen carbonate, and add 1 mL of potassium iodide TS: the solution does not show a red

color.

(2) To an amount of Fosfomycin Calcium for Syrup, equivalent to 40 mg (potency) of Fosfomycin Calcium Hydrate, add 10 mL of warm water, shake for 10 to 20 minutes, and collect the insoluble substances by filtration. Dissolve the substances in 3 mL of a solution of perchloric acid (1 in 4), add 2 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath for 10 minutes. After cooling, add 1 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, and allow to stand for 30 minutes: the solution shows a blue color.

(3) To an amount of Fosfomycin Calcium for Syrup, equivalent to 40 mg (potency) of Fosfomycin Calcium Hydrate, add 10 mL of warm water, shake for 10 to 20 minutes, and collect the insoluble substances by filtration. Dissolve the substances in 25 mL of water: the solution responds to the Qualitative Tests <*1.09*> (3) for calcium salt.

Loss on drying <*2.41*> Not more than 3.0% (2 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units <*6.02*> Fosfomycin Calcium for Syrup in single-dose packages meets the requirement of the Mass variation test.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Fosfomycin Calcium for Syrup is not less than 80%.

Start the test with an accurately weighed amount of Fosfomycin Calcium for Syrup, equivalent to about 0.5 g (potency) of Fosfomycin Calcium Hydrate, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Fosfomycin Phenethylammonium RS, equivalent to about 28 mg (potency), dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of fosfomycin in each solution.

Dissolution rate (%) with respect to the labeled amount of fosfomycin ($C_3H_7O_4P$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 1800$

M_S: Amount [mg (potency)] of Fosfomycin Phenethylammonium RS taken
M_T: Amount (g) of Fosfomycin Calcium for Syrup taken
C: Labeled amount [mg (potency)] of fosfomycin ($C_3H_7O_4P$) in 1 g

Operating conditions—

Detector: A conductivity detector.

Column: A polyetheretherketone column 4.6 mm in inside diameter and 7.5 cm in length, packed with quaternary ammonium group introducing hydrophilic vinyl polymer gel for liquid chromatography (6 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 10.5 g of citric acid monohydrate in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust so that the retention time of fosfomycin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fosfomycin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fosfomycin is not more than 2.0%.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

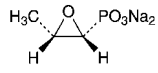
(i) Test organism, culture medium, agar media for seed and base layer, and standard solutions—Proceed as directed in the Assay under Fosfomycin Calcium Hydrate.

(ii) Sample solutions—Weigh accurately an amount of Fosfomycin Calcium for Syrup, equivalent to about 0.1 g (potency) of Fosfomycin Calcium Hydrate, dissolve in 0.05 mol/L tris buffer solution (pH 7.0) to make exactly 200 mL. Take exactly a suitable amount of this solution, add exactly 0.05 mol/L tris buffer solution (pH 7.0) to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Fosfomycin Sodium

ホスホマイシンナトリウム



$C_3H_5Na_2O_4P$: 182.02
Disodium (2*R*,3*S*)-3-methyloxiran-2-ylphosphonate
[*26016-99-9*]

Fosfomycin Sodium is the sodium salt of a substance having antibacterial activity produced by the growth of *Streptomyces fradiae* or by the chemical synthesis.

It contains not less than 725 μg (potency) and not more than 770 μg (potency) per mg, calculated on the anhydrous basis. The potency of Fosfomycin Sodium is expressed as mass (potency) of fosfomycin ($C_3H_7O_4P$: 138.06).

Description Fosfomycin Sodium occurs as a white crystalline powder.

It is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Identification **(1)** Determine the infrared absorption spectrum of Fosfomycin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ^1H spectrum of a solution of Fosfomycin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 300), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits

a double signal at around δ 1.5 ppm, a duple double signal at around δ 2.8 ppm, a multiple signal at around δ 3.3 ppm, and no signal at around δ 1.3 ppm.

(3) A solution of Fosfomycin Sodium (1 in 500) responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: -3.5 – $-5.5°$ (0.5 g calculated on the anhydrous bases, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.70 g of Fosfomycin Sodium in 10 mL of water: the pH of the solution is between 8.5 and 10.5.

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Sodium, add 40 mL of a solution of sodium periodate (107 in 10,000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 70 mg of potassium dihydrogen phosphate, proceed with this solution in the same manner as directed for the preparation of the sample stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the sample stock solution without using Fosfomycin Sodium, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the sample stock solution, the standard stock solution, and the blank stock solution, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, mix, and add water to make exactly 25 mL, and use these solutions as the sample solution, the standard solution, and the blank solution, respectively. After allowing these solutions to stand for 30 minutes at 20 ± 1°C, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as a blank, and determine the absorbances at 740 nm, A_T, A_S and A_B, of the sample solution, the standard solution and the blank solution: the content of phosphorus is 16.2 – 17.9%.

$$\text{Amount (mg) of phosphorus (P)} = M \times (A_T - A_B)/(A_S - A_B) \times 0.228$$

M: Amount (mg) of potassium dihydrogen phosphate taken

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Fosfomycin Sodium in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Fosfomycin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fosfomycin Sodium accordiong to Method 3, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 3.0% (0.2 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Proteus* sp. (MB838)

(ii) Culture medium—Mix 5.0 g of peptone, 3.0 g of meat extract, 2.0 g of yeast extract, and 15 g of agar in 1000 mL of water, sterilize, and use as the agar media for base layer and seed layer with the pH of between 6.5 and 6.6 after sterilization.

(iii) Seeded agar layer—Incubate the test organism on the slant of the agar medium for transferring test organisms at 37°C for 40 – 48 hours. Subcultures at least 3 times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organisms in a Roux bottle, incubate at 37°C for 40 – 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water, and use this as the stock suspension of test organism. The amount of the water to be added is adjust so that the percent transmission at 560 nm of the suspension diluted ten times with water is 17%. Keep the stock suspension at 10°C or below and use within 7 days. Add 1.0 – 2.0 mL of the stock suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the deeded agar layer.

(iv) Standard solutions—Weigh accurately an amount of Fosfomycin Phenethylammonium RS equivalent to about 20 mg (potency), dissolve in 0.05 mol/L tris buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.05 mol/L tris buffer solution (pH 7.0) to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(v) Sample solutions—Weigh accurately an amount of Fosfomycin Sodium equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L tris buffer solution (pH 7.0) to make exactly 50 mL. To exactly a suitable amount of this solution add 0.05 mol/L tris buffer solution (pH 7.0) to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Fosfomycin Sodium for Injection

注射用ホスホマイシンナトリウム

Fosfomycin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of fosfomycin ($C_3H_7O_4P$: 138.06).

Method of preparation Prepare as directed under Injections, with Fosfomycin Sodium.

Description Fosfomycin Sodium for Injection occurs as a white crystalline powder.

Identification **(1)** Dissolve about 0.1 g of Fosfomycin Sodium for Injection in 3 mL of a solution of perchloric acid (1 in 4), add 1 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath at 60°C for 30 minutes. After cooling, add 50 mL of water, neutralize with saturated sodium hydrogen carbonate solution, and add 1 mL of potassium iodide TS; the solution does not reveal a red color, while the blank solution reveals a red color.

(2) To 2 mL of a solution of Fosfomycin Sodium for Injection (1 in 250) add 1 mL of perchloric acid and 2 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath for 10 minutes. After cooling, add 1 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-

2-naphtol-4-sulfonic acid TS, and allow to stand for 30 minutes: a blue color develops.

(3) Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 0.1 g (potency) of Fosfomycin Sodium, in 50 mL of water. Perform the test with this solution as directed in the Identification (3) under Fosfomycin Sodium.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of Fosfomycin Sodium, in 20 mL of water is between 6.5 and 8.5.

Purity Clarity and color of solution—Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of Fosfomycin Sodium, in 10 mL of water: the solution is clear and colorless.

Water ⟨*2.48*⟩ Not more than 4.0% (0.1 g, coulometric titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.025 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, culture medium, seeded agar layer, and standard solutions—Proceed as directed in the Assay under Fosfomycin Sodium.

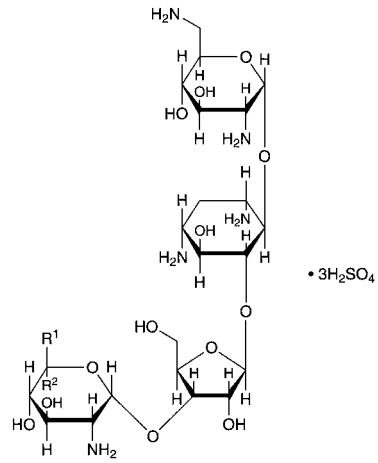
(ii) Sample solutions—Weigh accurately the mass of the contents of not less than 10 Fosfomycin Sodium for Injection. Weigh accurately an amount of the content, equivalent to about 20 mg (potency) of Fosfomycin Sodium, and dissolve in 0.05 mol/L tris buffer solution (pH 7.0) to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L tris buffer solution (pH 7.0) to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Fradiomycin Sulfate

Neomycin Sulfate

フラジオマイシン硫酸塩



Fradiomycin B : R^1=H R^2=CH_2NH_2

Fradiomycin C : R^1=CH_2NH_2 R^2=H

$C_{23}H_{46}N_6O_{13}.3H_2SO_4$: 908.88

Fradiomycin Sulfate B

2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)-[2,6-diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-β-D-ribofuranosyl-(1→5)]-2-deoxy-D-streptamine trisulfate

[*119-04-0*, Neomycin B]

Fradiomycin Sulfate C

2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→3)-β-D-ribofuranosyl-(1→5)]-2-deoxy-D-streptamine trisulfate

[*66-86-4*, Neomycin C]

[*1405-10-3*, Neomycin Sulfate]

Fradiomycin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Streptomyces fradiae*.

It, when dried, contains not less than 623 μg (potency) and not more than 740 μg (potency) per mg. The potency of Fradiomycin Sulfate is expressed as mass (potency) of fradiomycin ($C_{23}H_{46}N_6O_{13}$: 614.64).

Description Fradiomycin Sulfate occurs as a white to light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification (1) Dissolve 50 mg each of Fradiomycin Sulfate and Fradiomycin Sulfate RS in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the principal spot obtained from the sample solution and the spot from the standard solution show the same *R*f value.

(2) A solution of Fradiomycin Sulfate (1 in 20) responds

to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +53.5 – +59.0° (1 g calculated on the dried basis, water, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Fradiomycin Sulfate in 10 mL of water is between 5.0 and 7.5.

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Fradiomycin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fradiomycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.63 g of Fradiomycin Sulfate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the spot at around *R*f value 0.4 from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 8.0% (0.2 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Agar medium for seed and base layer

Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients and sterilize. Adjust the pH after sterilization to 7.8 – 8.0 with sodium hydroxide TS.

(iii) Standard solutions—Weigh accurately an amount of Fradiomycin Sulfate RS, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

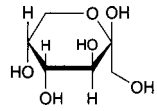
(iv) Sample solutions—Weigh accurately an amount of Fradiomycin Sulfate, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fructose

果糖



$C_6H_{12}O_6$: 180.16

β-D-Fructopyranose

[57-48-7]

Fructose, when dried, contains not less than 98.0% of fructose ($C_6H_{12}O_6$).

Description Fructose occurs as colorless to white, crystals or crystalline powder. It is odorless and has a sweet taste.

It is very soluble in water, sparingly soluble in ethanol (95) and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** Add 2 to 3 drops of a solution of Fructose (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.

(2) Determine the infrared absorption spectrum of Fructose as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 4.0 g of Fructose in 20 mL of water: the pH of the solution is between 4.0 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 25.0 g of Fructose in 50 mL of water: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS, and add water to make 10.0 mL. To 3.0 mL of the solution add water to make 50 mL.

(2) Acidity—Dissolve 5.0 g of Fructose in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS and 0.60 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <1.03>—Perform the test with 2.0 g of Fructose. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(4) Sulfate <1.14>—Perform the test with 2.0 g of Fructose. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Sulfite—Dissolve 0.5 g of Fructose in 5 mL of water, and add 0.25 mL of 0.02 mol/L iodine: the color of the solution is yellow.

(6) Heavy metals <1.07>—Proceed with 5.0 g of Fructose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(7) Calcium—Dissolve 0.5 g of Fructose in 5 mL of water, add 2 to 3 drops of ammonia TS and 1 mL of ammonium oxalate TS, and allow to stand for 1 minute: the solution is clear.

(8) Arsenic <*1.11*>—Dissolve 1.5 g of Fructose in 5 mL of water, heat with 5 mL of dilute sulfuric acid and 1 mL of bromine TS on a water bath for 5 minutes, concentrate to 5 mL, and cool. Perform the test with this solution as the test solution (not more than 1.3 ppm).

(9) 5-Hydroxymethylfurfurals—Dissolve 5.0 g of Fructose in 100 mL of water, and read the absorbance at 284 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.32.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 4 g of Fructose, previously dried, dissolve in 0.2 mL of ammonia TS and 80 mL of water, and after standing for 30 minutes add water to make exactly 100 mL, and determine the optical rotation, α_D, in a 100-mm cell at 20 ± 1°C as directed under Optical Rotation Determination <*2.49*>.

Amount (mg) of fructose ($C_6H_{12}O_6$) = $|\alpha_D| \times 1087.0$

Containers and storage Containers—Tight containers.

Fructose Injection

果糖注射液

Fructose Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of fructose ($C_6H_{12}O_6$: 180.16).

Method of preparation Prepare as directed under Injections, with Fructose. No preservative is added.

Description Fructose Injection is a colorless to pale yellow, clear liquid. It has a sweet taste.

Identification **(1)** Take a volume of Fructose Injection, equivalent to 1 g of Fructose, dilute with water or concentrate on a water bath to 20 mL, if necessary, and use this solution as the sample solution. Add 2 to 3 drops of the sample solution to 5 mL of boiling Fehling's TS: a red precipitate is produced.

(2) To 10 mL of the sample solution obtained in (1) add 0.1 g of resorcinol and 1 mL of hydrochloric acid, and warm in a water bath for 3 minutes: a red color develops.

pH <*2.54*> 3.0 – 6.5 In the case where the labeled concentration of the injection exceeds 5%, dilute to 5% with water before the test.

Purity **(1)** Heavy metals <*1.07*>—Take a volume of Fructose Injection, equivalent to 5.0 g of Fructose, and evaporate on a water bath to dryness. With the residue, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution.

(2) Arsenic <*1.11*>—Take a volume of Fructose Injection, equivalent to 1.5 g of Fructose, dilute with water or concentrate on a water bath to 5 mL, if necessary, and add 5 mL of dilute sulfuric acid and 1 mL of bromine TS. Proceed as directed in the purity (8) under Fructose.

Residue on ignition <*2.44*> Measure exactly a volume of Fructose Injection, equivalent to 2 g of Fructose, evaporate on a water bath to dryness, and perform the test: the residue weighs not more than 2 mg.

Bacterial endotoxins <*4.01*> Less than 0.5 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

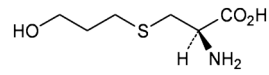
Assay Measure exactly a volume of Fructose Injection, equivalent to about 4 g of fructose ($C_6H_{12}O_6$), add 0.2 mL of ammonia TS, dilute with water to make exactly 100 mL, shake well, and after allowing to stand for 30 minutes, determine the optical rotation, α_D, in a 100-mm cell at 20 ± 1°C as directed under Optical Rotation Determination <*2.49*>.

Amount (mg) of fructose ($C_6H_{12}O_6$) = $|\alpha_D| \times 1087.0$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Fudosteine

フドステイン



$C_6H_{13}NO_3S$: 179.24

(2*R*)-2-Amino-3-(3-hydroxypropylsulfanyl)propanoic acid [*13189-98-5*]

Fudosteine, when dried, contains not less than 99.0% and not more than 101.0% of fudosteine ($C_6H_{13}NO_3S$).

Description Fudosteine occurs as white, crystals or crystalline powder.

It is freely soluble in water, slightly soluble in acetic acid (100), and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

Melting point: about 200°C (with decomposition).

Identification **(1)** To 5 mL of a solution of fudosteine (1 in 1000) add 2 mL of sodium hydroxide TS, shake well, add 0.3 mL of sodium pentacyanonitrosylferrate (III) TS, and shake well again. After allowing to stand at 40°C for 10 minutes, cool the solution in an ice bath for 2 minutes, add 2 mL of dilute hydrochloric acid, and shake: a red-orange color develops.

(2) Determine the infrared absorption spectrum of Fudosteine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −7.4 – −8.9° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity **(1)** Chloride <*1.03*>—Dissolve 0.20 g of Fudosteine in 10 mL of water and 20 mL of nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of nitric acid and water to make 50 mL (not more than 0.044%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Fudoste-

ine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Fudosteine according to Method 3, and perform the test (not more than 1 ppm).

(4) L-Cystine—Dissolve exactly 0.25 g of Fudosteine in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 25 mg of L-cystine in 2 mL of 1 mol/L hydrochloric acid TS, then add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the peak area of L-cystine obtained from the sample solution is not larger than the peak area of L-cystine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A solution of sodium 1-hexanesulfonate in diluted phosphoric acid (1 in 1000) (1 in 1250).

Flow rate: Adjust so that the retention time of fudosteine is about 8 minutes.

System suitability—

System performance: Dissolve 25 mg of L-cystine in 2 mL of 1 mol/L hydrochloric acid TS, add 25 mg of Fudosteine, and add the mobile phase to make 50 mL. Take 2.5 mL of this solution, add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, L-cystine and fudosteine are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of L-cystine is not more than 2.0%.

(5) Related substances—Dissolve 0.25 g of Fudosteine in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, pipet 2.5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than fudosteine obtained from the sample solution is not larger than the peak area of fudosteine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 55°C.

Mobile phase: Diluted phosphoric acid (1 in 1000).

Flow rate: Adjust so that the retention time of fudosteine is about 3 minutes.

Time span of measurement: About 10 times as long as the retention time of fudosteine, beginning after the peak of fudosteine.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fudosteine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fudosteine is not more than 2.0%.

Loss on drying <*2.41*>—Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*>—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Fudosteine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.92 mg of $C_6H_{13}NO_3S$

Containers and storage Containers—Well-closed containers.

Fudosteine Tablets

フドステイン錠

Fudosteine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of fudosteine ($C_6H_{13}NO_3S$: 179.24).

Method of preparation Prepare as directed under Tablets, with Fudosteine.

Identification Powder Fudostine Tablets. To a portion of the powder, equivalent to 88 mg of Fudosteine, add 10 mL of a mixture of water and methanol (1:1), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 90 mg of fudosteine for assay in 10 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2.5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a red-purple color and have the same *R*f value.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Fudosteine Tablets is not less than 85%.

Start the test with 1 tablet of Fudosteine Tablets, with-

draw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 55.6 μg of fudosteine ($C_6H_{13}NO_3S$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of fudosteine for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of fudosteine in each solution.

Dissolution rate (%) with respect to the labeled amount of fudosteine ($C_6H_{13}NO_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of fudosteine for assay taken
C: Labeled amount (mg) of fudosteine ($C_6H_{13}NO_3S$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fudosteine are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fudosteine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Fudosteine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 g of fudosteine ($C_6H_{13}NO_3S$), add 70 mL of the mobile phase, shake vigorously for 15 minutes, add the mobile phase to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of fudosteine for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fudosteine to that of the internal standard.

Amount (mg) of fudosteine ($C_6H_{13}NO_3S$)
$= M_S \times Q_T/Q_S \times 10$

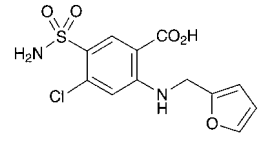
M_S: Amount (mg) of fudosteine for assay taken

Internal standard solution—A solution of L-methionine in the mobile phase (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: A solution of sodium 1-hexanesulfonate in diluted phosphoric acid (1 in 1000) (1 in 1250).
Flow rate: Adjust so that the retention time of fudosteine is about 8 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fudosteine and the internal standard are eluted in this order with the resolution between these peaks being not less than 12.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of fudosteine to that of the internal standard is not more than 1.0%.

Containers and storage containers—Tight containers.

Furosemide

フロセミド



$C_{12}H_{11}ClN_2O_5S$: 330.74
4-Chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid
[*54-31-9*]

Furosemide, when dried, contains not less than 98.0% and not more than 101.0% of furosemide ($C_{12}H_{11}ClN_2O_5S$).

Description Furosemide occurs as white, crystals or crystalline powder.
It is freely soluble in *N*,*N*-dimethylformamide, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in acetonitrile and in acetic acid (100), and practically insoluble in water.
It dissolves in dilute sodium hydroxide TS.
It is gradually colored by light.
Melting point: about 205°C (with decomposition).

Identification **(1)** Dissolve 25 mg of Furosemide in 10 mL of methanol. To 1 mL of this solution add 10 mL of 2 mol/L hydrochloric acid TS. Heat the solution under a reflux condenser on a water bath for 15 minutes, cool, and add 18 mL of sodium hydroxide TS to make weakly acidic: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines, producing a red to red-purple color.
(2) Determine the absorption spectrum of a solution of Furosemide in dilute sodium hydroxide TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Furosemide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(3) Determine the infrared absorption spectrum of Furosemide as directed in the potassium bromide disk

method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Furosemide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Furosemide in 10 mL of a solution of sodium hydroxide (1 in 50): the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 2.6 g of Furosemide in 90 mL of dilute sodium hydroxide TS, add 2 mL of nitric acid, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.020%).

(3) Sulfate <1.14>—To 20 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.030%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Furosemide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 25 mg of Furosemide in 25 mL of the dissolving solution, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the dissolving solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak appeared ahead of the peak of furosemide obtained from sample solution is not larger than 2/5 times the peak area of furosemide obtained from the standard solution, the area of each peak appeared behind the peak of furosemide is not larger than 1/4 times the peak area of furosemide from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of furosemide from the standard solution.

Dissolving solution—To 22 mL of acetic acid (100) add a mixture of water and acetonitrile (1:1) to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, tetrahydrofuran and acetic acid (100) (70:30:1).

Flow rate: Adjust so that the retention time of furosemide is about 18 minutes.

Time span of measurement: About 2.5 times as long as the retention time of furosemide, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the dissolving solution to make exactly 50 mL. Confirm that the peak area of furosemide obtained from 20 μL of this solution is equivalent to 3.2 to 4.8% of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of furosemide is not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of furosemide is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Furosemide, previously dried, dissolve in 50 mL of *N*,*N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank determination with a mixture of 50 mL of *N*,*N*-dimethylformamide and 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 33.07 mg of $C_{12}H_{11}ClN_2O_5S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Furosemide Injection

フロセミド注射液

Furosemide Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$: 330.74).

Method of preparation Prepare as directed under Injection, with Furosemide.

Description Furosemide Injection is a colorless, clear liquid.

Identification **(1)** To a volume of Furosemide Injection, equivalent to 2.5 mg of Furosemide, add 10 mL of 2 mol/L hydrochloric acid TS, heat under a reflux condenser on a water bath for 15 minutes. After cooling, render the solution slightly acid with 18 mL of sodium hydroxide TS: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines. The color of the solution is red to red-purple.

(2) To a volume of Furosemide Injection, equivalent to 20 mg of Furosemide, add water to make 100 mL. To 2 mL of this solution add 0.01 mol/L sodium hydroxide TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 227 nm and 231 nm, between 269 nm and 273 nm, and between 330 nm and 336 nm.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Pipet a volume of Furosemide Injection, equivalent to 40 mg of Furosemide, add 30 mL of acetone, shake well, and add acetone to make exactly 50 mL. Centrifuge this so-

lution, to 1.0 mL of the supernatant liquid add 3.0 mL of water, cool in a ice bath, add 3.0 mL of dilute hydrochloric acid and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. To this solution add 1.0 mL of ammonium amidosulfate TS, shake well, allow to stand for 3 minutes, add 1.0 mL of *N*,*N*-diethyl-*N'*-1-naphtylethylenediamine oxalate TS, shake well, and allow to stand for 5 minutes. Determine the absorbance of this solution at 530 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a solution, prepared in the same manner with 1.0 mL of acetone, as the blank: the absorbance is not more than 0.10.

Bacterial endotoxins <*4.01*> Less than 1.25 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Furosemide Injection, equivalent to about 20 mg of Furosemide ($C_{12}H_{11}ClN_2O_5S$), add water to make exactly 100 mL. Pipet 3 mL of this solution, add 0.01 mol/L sodium hydrochloride TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105°C for 4 hours, add 0.01 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.01 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 271 nm.

$$\text{Amount (mg) of furosemide } (C_{12}H_{11}ClN_2O_5S) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Furosemide RS taken

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Furosemide Tablets

フロセミド錠

Furosemide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$: 330.74).

Method of preparation Prepare as directed under Tablets, with Furosemide.

Identification (1) Shake well a quantity of powdered Furosemide Tablets, equivalent to 0.2 g of Furosemide, with 40 mL of acetone, and filter. To 0.5 mL of the filtrate add 10 mL of 2 mol/L hydrochloric acid TS, and heat under a reflux condenser on a water bath for 15 minutes. After cooling, add 18 mL of sodium hydroxide TS to make the solution slightly acidic: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines, producing a red to red-purple color.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 227 nm and 231 nm, between 269 nm and 273 nm, and between 330 nm and 336 nm.

Purity To a quantity of powdered Furosemide Tablets, equivalent to 40 mg of Furosemide, add about 30 mL of acetone, shake well, and add acetone to make exactly 50 mL. Centrifuge the solution, add 3.0 mL of water to 1.0 mL of the supernatant liquid, cool in ice, add 3.0 mL of dilute hydrochloric acid and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. Add 1.0 mL of ammonium amidosulfate TS, shake well, allow to stand for 3 minutes, add 1.0 mL of *N*,*N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS, shake well, and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared in the same manner with 1.0 mL of acetone as the blank: the absorbance at 530 nm is not more than 0.10.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Furosemide Tablets add a suitable amount of 0.05 mol/L sodium hydroxide TS, shake to disintegrate, then add 0.05 mol/L sodium hydroxide TS to make exactly V mL so that each mL contains about 0.4 mg of furosemide ($C_{12}H_{11}ClN_2O_5S$). Filter the solution, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of furosemide } (C_{12}H_{11}ClN_2O_5S) = M_S \times A_T/A_S \times V/50$$

M_S: Amount (mg) of Furosemide RS taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rates of a 20-mg tablet in 15 minutes and a 40-mg tablet in 30 minutes are not less than 80%.

Start the test with 1 tablet of Furosemide Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 10 μg of furosemide ($C_{12}H_{11}ClN_2O_5S$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105°C for 4 hours, and dissolve in 5 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 277 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Dissolution rate (\%) with respect to the labeled amount of furosemide } (C_{12}H_{11}ClN_2O_5S) = M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of Furosemide RS taken
C: Labeled amount (mg) of furosemide ($C_{12}H_{11}ClN_2O_5S$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Furosemide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 40 mg of furosemide

($C_{12}H_{11}ClN_2O_5S$), add about 70 mL of 0.05 mol/L sodium hydroxide TS, shake well, and add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide RS, previously dried at 105°C for 4 hours, and dissolve in 0.05 mol/L sodium hydroxide TS to make exactly 50 mL. Pipet 2 mL of this solution, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 271 nm as directed under the Ultraviolet-visible Spectrophotometry <*2.24*>.

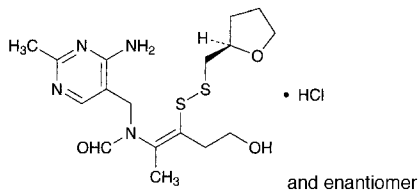
Amount (mg) of furosemide ($C_{12}H_{11}ClN_2O_5S$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of Furosemide RS taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Fursultiamine Hydrochloride

フルスルチアミン塩酸塩



$C_{17}H_{26}N_4O_3S_2.HCl$: 435.00
N-(4-Amino-2-methylpyrimidin-5-ylmethyl)-*N*-{(1*Z*)-4-hydroxy-1-methyl-2-[(2*RS*)-tetrahydrofuran-2-ylmethyldisulfanyl]but-1-en-1-yl}formamide monohydrochloride
[*804-30-8*, Fursultiamine]

Fursultiamine Hydrochloride contains not less than 98.5% of fursultiamine hydrochloride ($C_{17}H_{26}N_4O_3S_2.HCl$), calculated on the anhydrous basis.

Description Fursultiamine Hydrochloride occurs as white, crystals or crystalline powder. It is odorless or has a characteristic odor, and has a bitter taste.

It is freely soluble in water, in methanol and in ethanol (95).

It shows crystal polymorphism.

Identification **(1)** Dissolve 5 mg of Fursultiamine Hydrochloride in 6 mL of 0.1 mol/L hydrochloric acid TS, add 0.1 g of zinc powder, allow to stand for several minutes, and filter. To 3 mL of the filtrate, add 3 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS, then add 5 mL of 2-methyl-1-propanol, shake vigorously for 2 minutes, allow to stand to separate the 2-methyl-1-propanol layer, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. The fluorescence disappears by acidifying, and appears again by alkalifying.

(2) Determine the infrared absorption spectrum of Fursultiamine Hydrochloride, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Fursultiamine Hydrochloride RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. If any differences appear, dissolve the Fursultiamine Hydrochloride in water, evaporate the water, and dry the residue in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and repeat the test.

(3) A solution of Fursultiamine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Fursultiamine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Proceed with 1.5 g of Fursultiamine Hydrochloride, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Fursultiamine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Fursultiamine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following condition. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than fursultiamine from the sample solution is not larger than the peak area of fursultiamine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of fursultiamine from 10 μL of the standard solution is between 20 mm and 30 mm.

Time span of measurement: About 3 times as long as the retention time of fursultiamine.

Water <*2.48*> Not more than 5.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 55 mg each of Fursultiamine Hydrochloride and Fursultiamine Hydrochloride RS (previously determined the water <*2.48*> in the same manner as Fursultiamine Hydrochiloride) and dissolve each in 50 mL of water, and add exactly 10 mL each of the internal standard solution, then add water to make exactly 100 mL. To 8 mL each of the solution add water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fursultiamine to that of the internal standard.

Amount (mg) of fursultiamine hydrochloride ($C_{17}H_{26}N_4O_3S_2.HCl$)
$= M_S \times Q_T/Q_S$

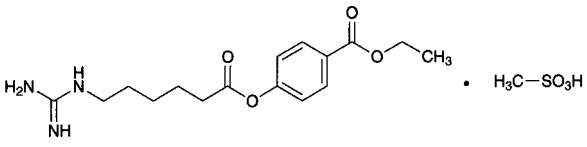
M_S: Amount (mg) of Fursultiamine Hydrochloride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl 4-aminobenzoate in ethanol (95) (3 in 400).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: Dissolve 1.01 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 675 mL of this solution add 325 mL of a mixture of methanol and acetonitrile (3:2).
Flow rate: Adjust so that the retention time of Fursultiamine is about 9 minutes.
Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions and calculate the resolution. Use a column giving elution of fursultiamine and the internal standard in this order with the resolution between these peaks being not less than 10.

Containers and storage Containers—Tight containers.

Gabexate Mesilate

ガベキサートメシル酸塩



$C_{16}H_{23}N_3O_4.CH_4O_3S$: 417.48
Ethyl 4-(6-guanidinohexanoyloxy)benzoate monomethanesulfonate
[*56974-61-9*]

Gabexate Mesilate, when dried, contains not less than 98.5% and not more than 101.0% of gabexate mesilate ($C_{16}H_{23}N_3O_4.CH_4O_3S$).

Description Gabexate Mesilate occurs as white, crystals or crystalline powder.
It is very soluble in water, freely soluble in ethanol (95).

Identification **(1)** To 4 mL of a solution of Gabexate Mesilate (1 in 2000) add 2 mL of 1-naphthol TS and 1 mL of diacetyl TS, and allow to stand for 10 minutes: a red color develops.
(2) Dissolve 1 g of Gabexate Mesilate in 5 mL of water, add 2 mL of sodium hydroxide TS, and heat in a water bath for 5 minutes. After cooling, add 2 mL of dilute nitric acid and 5 mL of ethanol (95), shake, add 5 drops of iron (III) chloride TS, and shake: a purple color develops.
(3) Determine the absorption spectrum of a solution of Gabexate Mesilate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Gabexate Mesilate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(4) A 0.1 g portion of Gabexate Mesilate responds to the Qualitative Tests <*1.09*> (1) for mesilate.

pH <*2.54*> Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the pH of the solution is between 4.7 and 5.7.

Melting point <*2.60*> 90 – 93°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the solution is clear and colorless.
(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Gabexate Mesilate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(3) Arsenic <*1.11*>—Dissolve 2.0 g of Gabexate Mesilate in 20 mL of 1 mol/L hydrochloric acid TS by heating in a water bath, and continue the heating for 20 minutes. After cooling, centrifuge, and use 10 mL of the supernatant liquid as the test solution. Perform the test (not more than 2 ppm).
(4) Ethyl parahydroxybenzoate—Weigh 50 mg of Gabexate Mesilate, previously dried, and dissolve in dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, dissolve 5.0 mg of ethyl parahydroxybenzoate in dilute ethanol to make exactly 100 mL. Pipet 1 mL of this solution, and add dilute ethanol to make exactly 20 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethyl parahydroxybenzoate to that of the internal standard: Q_T is not larger than Q_S.
Internal standard solution—A solution of butyl parahydroxybenzoate in dilute ethanol (1 in 5000).
Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
Proceed as directed in the system suitability in the Assay.
(5) Related substances—Dissolve 0.20 g of Gabexate Mesilate in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate until it has no acetic odor. Spray evenly a solution of 8-quinolinol in acetone (1 in 1000) on the plate, and after air-drying, spray evenly bromine-sodium hydroxide TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Gabexate Mesilate and Gabexate Mesilate RS, previously dried, and dissolve each in dilute ethanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate

the ratios, Q_T and Q_S, of the peak area of gabexate to that of the internal standard.

Amount (mg) of gabexate mesilate ($C_{16}H_{23}N_3O_4.CH_4O_3S$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Gabexate Mesilate RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in dilute ethanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, a solution of sodium lauryl sulfate (1 in 1000), a solution of sodium 1-heptane sulfonate (1 in 200) and acetic acid (100) (540:200:20:1).

Flow rate: Adjust so that the retention time of gabexate is about 13 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and gabexate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of gabexate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

β-Galactosidase (Aspergillus)

β-ガラクトシダーゼ（アスペルギルス）

[*9031-11-2*]

β-Galactosidase (Aspergillus) contains an enzyme produced by *Aspergillus oryzae*. It is an enzyme drug having lactose decomposition activity.

It contains 8,000 to 12,000 units per g.

Usually, it is diluted with a mixture of Maltose Hydrate and Dextrin, Maltose Hydrate and D-Mannitol, or Maltose Hydrate, Dextrin and D-Mannitol.

Description β-Galactosidase (Aspergillus) occurs as a white to light yellow powder.

It is slightly soluble in water with a turbidity, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 25 mg of β-Galactosidase (Aspergillus) in 100 mL of water, then to 1 mL of this solution add 9 mL of lactose substrate TS, and stand at 30°C for 10 minutes. To 1 mL of this solution add 6 mL of glucose detection TS, and stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.1 g of β-Galactosidase (Asperigillus) in 100 mL of water, and filter the solution if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Odor—β-Galactosidase (Aspergillus) has no any rancid odor.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of β-Galactosidase (Aspergillus) according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of β-Galactosidase (Aspergillus) according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 9.0% (0.5 g, in vacuum, 80°C, 4 hours).

Residue on ignition <*2.44*> Not more than 3% (0.5 g).

Nitrogen content Weigh accurately about 70 mg of β-Galactosidase (Aspergillus), and perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is between 0.5% and 5.0%, calculated on the dried basis.

Assay (i) Substrate solution—Dissolve 0.172 g of 2-nitrophenyl-β-D-galactopyranoside in disodium hydrogenphosphate-citric acid buffer solution (pH 4.5) to make 100 mL.

(ii) Procedure—Weigh accurately about 25 mg of β-Galactosidase (Aspergillus), dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Take exactly 3.5 mL of the substrate solution, stand at 30 ± 0.1°C for 5 minutes, add exactly 0.5 mL of the sample solution, immediately mix, and stand at 30 ± 0.1°C for exactly 10 minutes, then add exactly 1 mL of sodium carbonate TS and mix immediately. Perfoum the test as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbance, A_1, of this solution at 420 nm using water as the control. Separately, take exactly 3.5 mL of the substrate solution, add exactly 1 mL of sodium carbonate TS and mix, then add exactly 0.5 mL of the sample solution and mix. Determine the absorbance, A_2, of this solution in the same manner as above.

Units per g of β-Galactosidase (Aspergillus) $= 1/M \times (A_1 - A_2)/0.917 \times 1/0.5 \times 1/10$

0.917: Absorbance of 1 μmol/5 mL of *o*-nitrophenol

M: Amount (g) of β-Galactosidase (Aspergillus) in the sample solution per mL

Unit: One unit indicates an amount of the enzyme which decomposes 1 μmol of 2-nitrophenyl-β-D-galactopyranoside in 1 minute under the above conditions.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

β-Galactosidase (Penicillium)

β-ガラクトシダーゼ（ペニシリウム）

[*9031-11-2*]

β-Galactosidase (Penicillium) contains an enzyme, having lactose decomposition activity, produced by *Penicillium multicolor*.

It contains not less than 8500 units and not more than 11,500 units in each g.

Usually, it is diluted with D-Mannitol.

Description β-Galactosidase (Penicillium) occurs as a white to pale yellowish white, crystalline powder or powder.

It is soluble in water with a turbidity, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification **(1)** Dissolve 0.05 g of β-Galactosidase (Penicillium) in 100 mL of water, then to 0.2 mL of this solution add 0.2 mL of lactose substrate TS for β-galactosidase (penicillium), and allow to stand at 30°C for 10 minutes. To this solution add 3 mL of glucose detection TS for penicillium origin β-galactosidase, and allow to stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.15 g of β-Galactosidase (Penicillium) in 100 mL of water, filter if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 278 nm and 282 nm.

Purity **(1)** Odor—β-Galactosidase (Penicillium) has no any rancid odor.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of β-Galactosidase (Penicillium) according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of β-Galactosidase (Penicillium) according to Method 3, and perform the test (not more than 2 ppm).

(4) Nitrogen—Weigh accurately about 0.1 g of β-Galactosidase (Penicillium), and perform the test as directed under Nitrogen Determination <*1.08*>: not more than 3 mg of nitrogen (N: 14.01) is found for each labeled 1000 Units.

(5) Protein contaminants—Dissolve 0.15 g of β-Galactosidase (Penicillium) in 4 mL of water, and use this solution as the sample solution. Perform the test with 15 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak having retention time of about 19 minutes is not more than 75% of the total area of all peaks, and the areas of peaks other than the peaks having retention times of about 3, 16 and 19 minutes are not more than 15% of the total area of all peaks.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 7.5 mm in inside diameter and about 75 mm in length, packed with strongly acidic ion-exchange resin for liquid chromatography of sulfopropyl group-binding hydrophilic polymer (10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A solution obtained by dissolving 2.83 g of sodium acetate in 1000 mL of water, and adjusting to pH 4.5 with acetic acid (100) (mobile phase A), and a solution obtained by dissolving 29.2 g of sodium chloride in 1000 mL of mobile phase A (mobile phase B).

Flow system: Adjust a linear concentration gradient from the mobile phase A to the mobile phase B immediately after injection of the sample so that the retention times of non-retaining protein and the enzyme protein are about 3 minutes and 19 minutes, respectively, when the flow runs 0.8 mL per minute, and then continue the running of the mobile phase B.

Selection of column: Dissolve 15 mg of β-lactoglobulin in 4.5 mL of water, add 0.5 mL of a solution of cytosine (1 in 5000), and use this solution as the column-selecting solution. Proceed with 15 μL of the column-selecting solution under the above operating conditions, and calculate the resolution. Use a column giving elution of cytosine and β-lactoglobulin in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of β-lactoglobulin from 15 μL of the column-selecting solution is between 5 cm and 14 cm.

Time span of measurement: About 1.4 times as long as the retention time of β-lactoglobulin.

Loss on drying <*2.41*> Not more than 5.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <*2.44*> Not more than 2% (1 g).

Assay (i) Substrate solution—Dissolve 0.603 g of 2-nitrophenyl-β-D-galactopyranoside in disodium hydrogen phosphate-citric acid buffer solution for penicillium origin β-galactosidase (pH 4.5) to make 100 mL.

(ii) Procedure—Weigh accurately about 0.15 g of β-Galactosidase (Penicillium), dissolve in water with thorough shaking to make exactly 100 mL, and allow to stand at room temperature for an hour. Pipet 2 mL of this solution, add disodium hydrogen phosphate-citric acid buffer solution for penicillium origin β-galactosidase (pH 4.5) to make exactly 100 mL, and use this solution as the sample solution. Transfer exactly 0.5 mL of the sample solution to a test tube, stand at 30 ± 0.1°C for 10 minutes, add exactly 0.5 mL of the substrate solution previously kept at 30 ± 0.1°C, then mix immediately, and stand at 30 ± 0.1°C for exactly 10 minutes. Then add exactly 1 mL of sodium carbonate TS, mix immediately to stop the reaction. To this solution add exactly 8 mL of water, mix, and use as the colored sample solution. Separately, pipet 0.5 mL of disodium hydrogen phosphate-citric acid buffer solution for penicillium origin β-galactosidase (pH 4.5), then proceed in the same manner as the sample solution, and use the solution so obtained as the colored blank solution. Perform the test with the colored sample solution and the colored blank solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank, and determine the absorbances, A_T and A_B, at 420 nm.

$$\text{Units per g of } \beta\text{-Galactosidase (Penicillium)} = 1/M \times (A_T - A_B)/0.459 \times 1/10$$

0.459: Absorbance of 1 μmol/10 mL of *o*-nitrophenol

M: Amount (g) of β-Galactosidase (Penicillium) in 0.5 mL of the sample solution

Unit: One unit indicates an amount of the enzyme which decomposes 1 μmol of 2-nitrophenyl-β-D-galactopyranoside in 1 minute under the above conditions.

Containers and storage Containers—Tight containers.

Gallium (^{67}Ga) Citrate Injection

クエン酸ガリウム（^{67}Ga）注射液

Gallium (^{67}Ga) Citrate Injection is an aqueous injection containing gallium-67 (^{67}Ga) in the form of gallium citrate.

It conforms to the requirements of Gallium (^{67}Ga) Citrate Injection in the Minimum Requirements for

Radiopharmaceuticals.

Test for Extractable volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Gallium (^{67}Ga) Citrate Injection is a clear, colorless or light red liquid.

Gas Gangrene Antitoxin, Equine

ガスえそウマ抗毒素

Gas Gangrene Antitoxin, Equine, is a liquid for injection containing *Clostridium perfringens* (*C. welchii*) Type A antitoxin, *Clostridium septicum* (*Vibrion septique*) antitoxin and *Clostridium oedematiens* (*C. novyi*) antitoxin in immunoglobulin of horse origin.

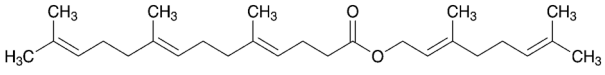
It may contain also *Clostridium histolyticum* antitoxin.

It conforms to the requirements of Gas Gangrene Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Gas Gangrene Antitoxin, Equine, is a colorless to light yellow-brown, clear liquid or a slightly whitish turbid liquid.

Gefarnate

ゲファルナート



$C_{27}H_{44}O_2$: 400.64

(2*E*)-3,7-Dimethylocta-2,6-dienyl(4*E*,8*E*)-5,9,13-trimethyltetradeca-4,8,12-trienoate

[*51-77-4*, 4*E* isomer]

Gefarnate is a mixture of 4*E* geometrical isomer.

It contains not less than 98.0% and not more than 101.0% of gefarnate ($C_{27}H_{44}O_2$).

Description Gefarnate is a light yellow to yellow, clear oily liquid.

It is miscible with acetonitrile, with ethanol (99.5) and with cyclohexane.

It is practically insoluble in water.

Identification Determine the infrared absorption spectrum of Gefarnate as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Gefarnate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <*2.56*> d_{20}^{20}: 0.906 – 0.914

Purity **(1)** Acidity—To 1.0 g of Gefarnate add 30 mL of neutralized ethanol. To this solution add 1 drop of phenolphthalein TS and 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Gefarnate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Use a solution of Gefarnate in acetonitrile (1 in 500) as the sample solution. Pipet 2 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of gefarnate obtained from the sample solution is not larger than 1/2 times the peak area of gefarnate obtained from the standard solution, and the total area of the peaks other than the peak of gefarnate from the sample solution is not larger than the peak area of gefarnate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of gefarnate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, add acetonitrile to make exactly 20 mL. Confirm that the peak area of gefarnate obtained from 2 μL of this solution is equivalent to 7 to 13% of that of gefarnate obtained from 2 μL of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of gefarnate are not less than 4000, and between 0.9 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gefarnate is not more than 1.0%.

Isomer ratio To 1 mL of Gefarnate add 100 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with 4 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having the retention time of about 37 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_a/(A_a + A_b)$ is between 0.2 and 0.3.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 160 cm in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 5% on acid-treated and silanized siliceous earth for gas chromatography (149 to 177 μm in particle diameter).

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the reaction time of the peak showing earlier elution of the two peaks of gefarnate is about 35 minutes.

System suitability—

System performance: When the procedure is run with 4 μL of the sample solution under the above conditions: the resolution between the two peaks of gefarnate is not less than 1.0.

System repeatability: When the test is repeated 6 times with 4 μL of the sample solution under the above operating conditions: the relative standard deviation of the peak area of gefarnate with the shorter retention time of the two peaks of gefarnate is not more than 2.0%.

Assay Weigh accurately about 50 mg each of Gefarnate and Gefarnate RS, add exactly 5 mL of the internal standard solution and 20 mL of acetonitrile, and use these solutions as the sample solution and standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of gefarnate to that of the internal standard.

$$\text{Amount (mg) of gefarnate } (C_{27}H_{44}O_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Gefarnate RS taken

Internal standard solution—A solution of tris (4-*t*-butylphenyl) phosphate in acetonitrile (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with phenylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile for liquid chromatography, water and phosphoric acid (700:300:1).

Flow rate: Adjust so that the retention time of gefarnate is about 19 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and gefarnate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of gefarnate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and under nitrogen atmosphere.

Gelatin

ゼラチン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Gelatin is a purified protein obtained from collagen of animals by partial alkaline and/or acid hydrolysis, or by thermal hydrolysis. The hydrolysis leads to gelling or non-gelling grades.

It is the gelling grade.

The label states the gel strength (Bloom value).

◆**Description** Gelatin occurs as colorless or white to light yellow-brown sheets, shreds, granules or powder.

It is freely soluble in hot water, and practically insoluble in ethanol (95).

It does not dissolve in water, but slowly swells and softens when immersed in it, gradually absorbing water 5 to 10 times its own mass.

Gelatin derived from an acid-treated collagen exhibits an isoelectric point between pH 7.0 and 9.0, and Gelatin derived from an alkali-treated collagen exhibits an isoelectric point between pH 4.5 and 5.0.◆

Identification **(1)** Dissolve 1.00 g of Gelatin in freshly boiled and cooled water at about 55°C to make 100 mL, and use this solution as the sample solution. To 2 mL of the sample solution keeping at about 55°C add 0.05 mL of copper (II) sulfate TS. Mix and add 0.5 mL of 2 mol/L sodium hydroxide TS: a violet color is produced.

(2) In a test tube about 15 mm in internal diameter, place 0.5 g of Gelatin, add 10 mL of water, and allow to stand for 10 minutes. Heat at 60°C for 15 minutes, then keep the tube upright at 0°C for 6 hours, and invert the tube: the contents do not flow out immediately.

Gel strength (Bloom value) Determine the mass (g) necessary to produce the force which, applied to a plunger 12.7 mm in diameter, makes a depression 4 mm deep in a gel having a concentration of 6.67% and matured at 10°C.

(i) Apparatus Texture analyzer or gelometer with a cylindrical piston 12.7 ± 0.1 mm in diameter with a plane pressure surface and a sharp bottom edge, and with a bottle 59 ± 1 mm in internal diameter and 85 mm high (jelly cup).

(ii) Procedure Place 7.5 g of Gelatin in a jelly cup, add 105 mL of water, close the cup, and allow to stand for 1 to 4 hours. Heat in a water bath at 65 ± 2°C for 15 minutes. While heating, stir gently with a glass rod. Ensure that the solution is uniform and any condensed water on the inner walls of the cup is incorporated. Allow to cool at room temperature for 15 minutes and transfer the cup to a thermostatically controlled bath at 10.0 ± 0.1°C, and fitted with a device to ensure that the platform on which the cup stands is perfectly horizontal. Close the cup, and allow to stand for 17 ± 1 hours. Remove the sample cup from the bath and quickly wipe the water from the exterior of the cup. Center the cup on the platform of the apparatus so that the plunger contacts the sample as nearly at its midpoint as possible, and start the measurement with 4 mm depression distance and 0.5 mm per second test speed: 80 to 120% of the labeled nominal value.

pH <*2.54*> pH at 55°C of the sample solution obtained in Identification (1) is 3.8 – 7.6.

Purity ◆**(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of Gelatin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).◆

(2) Iron—To 5.00 g of Gelatin, in a glass-stoppered flask, add 10 mL of hydrochloric acid, close the flask, and heat in a water bath at 75 – 80°C for 2 hours. If necessary for proper solubilization, the gelatin may be allowed to swell after addition of the acid and before heating, the heating time may be prolonged and a higher temperature may be used. After cooling, adjust the content of the flask to 100.0 g with water, and use this solution as the sample solution. Separately, place 5.00 g each of Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add 10 mL, 20 mL and 30 mL of Standard Iron Solution (2) for Atomic Absorption Spectrophotometry exactly to each flask separately. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. The amount of the standard solution to be added may be adjusted according to the sensitivity of the instrument to be used. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine the content of iron: not more than 30 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Iron hollow cathode lamp.
Wavelength: 248.3 nm.

(3) Chromium—Use the sample solution obtained in (2) as the sample solution. Separately, place 5.00 g each of Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add 0.25 mL, 0.50 mL and 0.75 mL of Standard Chromium Solution for Atomic Absorption Spectrophotometry exactly to each flask separately. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. The amount of the standard solution to be added may be adjusted according to the sensitivity of the instrument to be used. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine the content of chromium: not more than 10 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Chromium hollow cathode lamp.
Wavelength: 357.9 nm.

(4) Zinc—Use the sample solution obtained in (2) as the sample solution. Separately, place 5.00 g each of Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add 7.5 mL, 15 mL and 22.5 mL of Standard Zinc Solution for Atomic Absorption Spectrophotometry exactly to each flask separately. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. The amount of the standard solution to be added may be adjusted according to the sensitivity of the instrument to be used. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine the content of zinc: not more than 30 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.

◆**(5)** Arsenic <*1.11*>—Take 15.0 g of Gelatin in a flask, add 60 mL of diluted hydrochloric acid (1 in 5), and dissolve by heating. Add 15 mL of bromine TS, heat until the excess of bromine is expelled, neutralize with ammonia TS, add 1.5 g of disodium hydrogen phosphate dodecahydrate, and allow to cool. To this solution add 30 mL of magnesia TS, allow to stand for 1 hour, and collect the precipitates. Wash the precipitates with five 10-mL portions of diluted ammonia TS (1 in 4), and dissolve in diluted hydrochloric acid (1 in 4) to make exactly 50 mL. Perform the test with 5 mL of this solution: the solution has no more color than the following color standard.

Color standard: Proceed with 15 mL of Standard Arsenic Solution, instead of Gelatin, in the same manner (not more than 1 ppm).◆

(6) Peroxides—

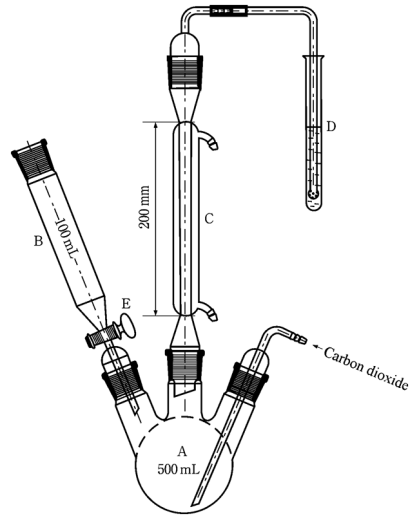
(i) Enzyme reaction: Peroxidase transfers oxygen from peroxides to an organic redox indicator which is converted to a blue oxidation product. The intensity of the color obtained is proportional to the quantity of peroxide and can be compared with a color scale provided with the test strips, to determine the peroxide concentration.

(ii) Procedure: Weigh 20.0 ± 0.1 g of Gelatin in a beaker, add 80.0 ± 0.2 mL of water, and stir to moisten all the gelatin. Allow to stand at room temperature for 1 – 3 hours. Cover the beaker with a watch-glass, and heat the beaker for 20 ± 5 minutes in a water bath at 65 ± 2°C for dissolving the sample. Stir the contents of the beaker with a glass rod to achieve a homogeneous solution, and use this as the sample solution. Dip a peroxide test strip for 1 second into the sample solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid, and compare the reaction zone after 15 seconds with the color scale provided. Multiply the concentration read from the color scale by a factor of 5 to calculate the concentration of peroxide in the test substance: not more than 10 ppm.

(iii) Suitability test: To exactly 10 mL of Standard Hydrogen Peroxide Solution add water to make exactly 300 mL. Pipet 2 mL of this solution, add water to make exactly 1000 mL (2 ppm). Dip a peroxide test strip for 1 second into this solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the color of the reaction zone after 15 seconds with the color scale: the color of the zone is equivalent to 2 ppm of the color scale.

(7) Sulfur dioxide—

(i) Apparatus: Use as shown in the figure.



A: Three-necked round-bottomed flask (500 mL)
B: Cylindrical dropping funnel (100 mL)
C: Condenser
D: Test tube
E: Tap

(ii) Procedure: Introduce 150 mL of water into the three-necked round-bottomed flask and pass carbon dioxide through the whole system at a rate of 100 mL per minute. Place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test tube. After 15 minutes, remove the cylindrical dropping funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the three-necked round-bottomed flask about 25.0 g of Gelatin with the aid of 100 mL of water. Pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the three-necked round-bottomed flask ◆and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide◆, and boil the mixture for 1 hour. Transfer the contents of the test tube with the aid of a little water to a 200 mL wide-necked conical flask. Heat the flask in a water bath for 15 minutes and cool. Add 0.1 mL of bromophenol blue TS and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make

any necessary correction. Calculate the amount of sulfur dioxide from the following expression: it is not more than 50 ppm.

Amount (ppm) of sulfur dioxide = $V/M \times 1000 \times 3.203$

M: Amount (g) of Gelatin taken
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Conductivity ⟨*2.51*⟩ Perform the test at 30 ± 1.0°C with the sample solution obtained in Identification (1), without temperature compensation: not more than 1 $mS \cdot cm^{-1}$.

Loss on drying ⟨*2.41*⟩ Not more than 15.0% (5 g, 105°C, 16 hours).

Microbial limit ⟨*4.05*⟩ The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively. *Escherichia coli* and *Salmonella* are not observed.

Containers and storage ◆Containers—Tight containers.◆
Storage—Protect from heat and moisture.

Purified Gelatin

精製ゼラチン

Purified Gelatin is a purified protein obtained from collagen of animals by partial alkaline and/or acid hydrolysis, and/or enzymatic hydrolysis, or by thermal hydrolysis. The hydrolysis leads to gelling or non-gelling grades.

The label states the gel strength (Bloom value) for the gelling grade, and that it is a non-gelling grade for the non-gelling grade.

Description Purified Gelatin occurs as colorless or white to light yellow-brown sheets, shreds, granules or powder.

It is very soluble in hot water, and practically insoluble in ethanol (95).

The gelling grade does not dissolve in water. It slowly swells and softens when immersed in water, and absorbs water 5 to 10 times its own mass. The non-gelling grade is freely soluble in water.

Identification **(1)** To 5 mL of a solution of Purified Gelatin (1 in 100) add 2,4,6-trinitrophenol TS dropwise: a precipitate is formed.

(2) To 5 mL of a solution of Purified Gelatin (1 in 5000) add tannic acid TS dropwise: the solution becomes turbid.

(3) In a test tube about 15 mm in internal diameter, place 0.5 g of Purified Gelatin, add 10 mL of water, and allow to stand for 10 minutes. Heat at 60°C for 15 minutes, then keep the tube upright in cold water for 6 hours, and invert the tube: the contents do not flow out immediately. In case of the non-gelling grade the contents flow out immediately.

Gel strength (Bloom value) Apply to the gelling grade. Determine the mass (g) necessary to produce the force which, applied to a plunger 12.7 mm in diameter, makes a depression 4 mm deep in the surface of the gel having a concentration of 6.67% and matured at 10°C.

(i) Apparatus, instruments Texture analyzer or gelometer with a cylindrical piston 12.7 ± 0.1 mm in diameter with a plane bottom and a sharp bottom edge, and with a cup 59 ± 1 mm in internal diameter and 85 mm high (jelly cup).

(ii) Procedure Place 7.5 g of Purified Gelatin in a jelly cup, add 105 mL of water, close the cup, and allow to stand for 1 to 4 hours. Heat in a water bath at 65 ± 2°C for 15 minutes. While heating, stir gently with a glass rod. Incorporate any condensed water on the inner wall of the cup into the solution, and ensure that the solution is uniform. Allow to cool at room temperature for 15 minutes and transfer the cup to a thermostatically controlled bath at 10.0 ± 0.1°C, and fitted with a device to ensure that the platform on which the cup stands is perfectly horizontal. Close the cup, and allow to stand for 17 ± 1 hours. Remove the sample cup from the bath and quickly wipe the water from the exterior of the cup. Put the cup on the platform of the apparatus so that the tip of plunger contacts the sample as nearly at its midpoint as possible, and start the measurement with 4 mm depression distance and 0.5 mm per second test speed: 80 to 120% of the labeled nominal value.

pH ⟨*2.54*⟩ Dissolve 1.00 g of Purified Gelatin in freshly boiled water and kept at about 55°C, to make 100 mL. pH at 55°C of this solution is 3.8 – 9.0.

Purity **(1)** Heavy metals⟨*1.07*⟩—Proceed with 1.0 g of Purified Gelatin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Iron—To 5.00 g of Purified Gelatin, in a glass-stoppered flask, add 10 mL of hydrochloric acid, close the flask, and place in a water bath at 75 – 80°C for 2 hours. If necessary for proper solubilization, the gelatin may be allowed to swell after addition of the acid and the heating time may be prolonged or a higher temperature may be used. After cooling, adjust the content of the flask to 100.0 g with water, and use this solution as the sample solution. Separately, place 5.00 g each of Purified Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add exactly 10 mL, 20 mL and 30 mL of Standard Iron Solution (2) for Atomic Absorption Spectrophotometry to each flask, respectively. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. The volume of Standard Iron Solution may be adjusted appropriately according to the sensitivity of the instrument to be used. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry⟨*2.23*⟩ according to the following conditions, and determine the content of iron: not more than 30 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Iron hollow cathode lamp.
Wavelength: 248.3 nm.

(3) Chromium—Use the sample solution obtained in (2) as the sample solution. Separately, place 5.00 g each of Purified Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add exactly 0.25 mL, 0.50 mL and 0.75 mL of Standard Chromium Solution for Atomic Absorption Spectrophotometry to each flask, respectively. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. The volume of Standard Iron Solution may be adjusted appropriately according to the sensitivity of the instrument to be used. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions, and determine the content of chromium: not more than 10 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Chromium hollow cathode lamp.

Wavelength: 357.9 nm.

(4) Zinc—Use the sample solution obtained in (2) as the sample solution. Separately, place 5.00 g each of Purified Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add exactly 7.5 mL, 15 mL and 22.5 mL of Standard Zinc Solution for Atomic Absorption Spectrophotometry to each flask, respectively. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. The volume of Standard Iron Solution may be adjusted appropriately according to the sensitivity of the instrument to be used. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry〈*2.23*〉 according to the following conditions, and determine the content of zinc: not more than 30 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.

(5) Arsenic〈*1.11*〉—Place 15.0 g of Purified Gelatin in a flask, add 60 mL of diluted hydrochloric acid (1 in 5), and dissolve by heating. Add 15 mL of bromine TS, heat until the excess of bromine is expelled, neutralize with ammonia TS, add 1.5 g of disodium hydrogen phosphate dodecahydrate, and allow to cool. To this solution add 30 mL of magnesia TS, allow to stand for 1 hour, and collect the precipitates. Wash the precipitates with five 10-mL portions of diluted ammonia TS (1 in 4), and dissolve in diluted hydrochloric acid (1 in 4) to make exactly 50 mL. Perform the test with 5 mL of this solution: the solution has no more color than the following color standard.

Color Standard: Proceed with 12 mL of Standard Arsenic Solution, instead of Purified Gelatin, in the same manner (not more than 0.8 ppm).

(6) Peroxides—

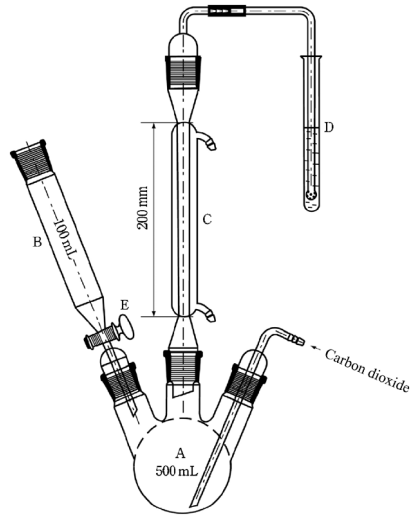
(i) Enzyme reaction: Peroxidase transfers oxygen atom at on from peroxides to an organic redox indicator which is converted to a blue oxidized form. The intensity of the color obtained is proportional to the quantity of peroxide. The peroxide concentration can be determined by comparing it with the color scale provided with the test strips employing this reaction.

(ii) Procedure: Weigh 20.0 ± 0.1 g of Purified Gelatin in a beaker, add 80.0 ± 0.2 mL of water, and stir to moisten all the gelatin. Allow to stand at room temperature for 1—3 hours. Cover the beaker with a watch-glass, and heat the beaker for 20 ± 5 minutes in a water bath at 65 ± 2°C to dissolve the sample. Stir the contents of the beaker with a glass rod to achieve a homogeneous solution, and use this as the sample solution. Dip a peroxide test strip for 1 second into the sample solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid, and compare the reaction zone after 15 seconds with the color scale provided. Multiply the concentration read from the color scale by a factor of 5 to calculate the concentration of peroxide in the test substance: not more than 10 ppm.

(iii) Sensitivity: To exactly 10 mL of Standard Hydrogen Peroxide Solution add water to make exactly 300 mL. Pipet exactly 2 mL of this solution, add water to make exactly 1000 mL (2 ppm). Dip a peroxide test strip for 1 second into this solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the color of the reaction zone after 15 seconds with the color scale: the color of the zone is equivalent to 2 ppm of the color scale.

(7) Sulfur dioxide—

(i) Apparatus: Use as shown in the figure.



A: Three-necked round-bottomed flask (500 mL)
B: Cylindrical dropping funnel (100 mL)
C: Condenser
D: Test tube
E: Tap

(ii) Procedure: Introduce 150 mL of water into the three-necked round-bottomed flask and pass carbon dioxide through the whole system at a rate of 100 mL per minute. Place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test tube. After 15 minutes, remove the cylindrical dropping funnel from the flask without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25.0 g of Purified Gelatin with the aid of 100 mL of water. Pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide, and boil the mixture for 1 hour. Remove the test tube, and transfer the contents of the test tube to a 200-mL wide-necked conical flask, wash the test tube with a small amount of water, and add the washing to the conical flask. Heat the flask in a water bath for 15 minutes and cool. Add 0.1 mL of bromophenol blue TS, and titrate 〈*2.50*〉 with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination in the same manner and make any necessary correction. Calculate the amount of sulfur dioxide from the following expression: it is not more than 20 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of Purified Gelatin taken
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Conductivity 〈*2.51*〉 Dissolve 1.00 g of Purified Gelatin in freshly boiled water and kept at about 55°C, to make 100 mL. Perform the test at 30 ± 1.0°C with this solution, without temperature compensation: not more than 1 $mS \cdot cm^{-1}$.

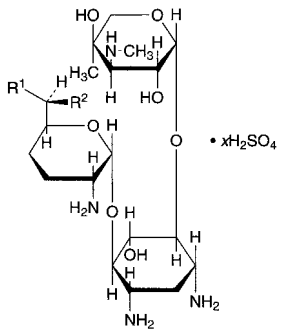
Loss on drying 〈*2.41*〉 Not more than 15.0% (5 g, 105°C, 16 hours).

Microbial limit 〈*4.05*〉 The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively. *Escherichia coli* and *Salmonella* are not observed.

Containers and storage Containers—Tight containers.
Storage—Protect from heat and moisture.

Gentamicin Sulfate

ゲンタマイシン硫酸塩



Gentamicin Sulfate C_1 : $R^1 = CH_3$ $R^2 = NHCH_3$
Gentamicin Sulfate C_2 : $R^1 = CH_3$ $R^2 = NH_2$
Gentamicin Sulfate C_{1a} : $R^1 = H$ $R^2 = NH_2$

Gentamicin Sulfate C_1 (6*R*)-2-Amino-2,3,4,6-tetradeoxy-6-methylamino-6-methyl-α-D-*erythro*-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine sulfate
Gentamicin Sulfate C_2 (6*R*)-2,6-Diamino-2,3,4,6-tetradeoxy-6-methyl-α-D-*erythro*-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine sulfate
Gentamicin Sulfate C_{1a} 2,6-Diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine sulfate
[*1405-41-0*, Gentamicin Sulfate]

Gentamicin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Micromonospora purpurea* or *Micromonospora echinospora*.

It contains not less than 590 μg (potency) and not more than 775 μg (potency) per mg, calculated on the dried basis. The potency of Gentamicin Sulfate is expressed as mass (potency) of gentamicin C_1 ($C_{21}H_{43}N_5O_7$: 477.60).

Description Gentamicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 50 mg each of Gentamicin Sulfate and Gentamicin Sulfate RS in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², and without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapors: three principal spots obtained from the sample solution are the same with the corresponding spots obtained from the standard solution in color tone and the *R*f value, respectively.

(2) Dissolve 50 mg of Gentamicin Sulfate in 5 mL of water, and add 0.5 mL of barium chloride TS: a white precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{25}$: +107 – +121° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 0.20 g of Gentamicin Sulfate in 5 mL of water is between 3.5 and 5.5.

Content ratio of the active principle Dissolve 50 mg of Gentamicin Sulfate in water to make 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapor. Determine the integral absorbances, A_a, A_b and A_c, of the colored spots of gentamicin C_1 (*R*f value: about 0.3), gentamicin C_2 (*R*f value: about 0.2) and gentamicin C_{1a} (*R*f value: about 0.1), respectively, by a densitometer (wavelength: 450 nm) while covering the plate with a glass plate, and calculate these amounts by the following formulae: gentamicin C_1 is between 25% and 55%, gentamicin C_2 is between 25% and 50%, and gentamicin C_{1a} is between 5% and 30%.

$$\text{Amount (\%) of gentamicin } C_1 = A_a/(A_a + 1.35A_b + A_c) \times 100$$

$$\text{Amount (\%) of gentamicin } C_2 = 1.35A_b/(A_a + 1.35A_b + A_c) \times 100$$

$$\text{Amount (\%) of gentamicin } C_{1a} = A_c/(A_a + 1.35A_b + A_c) \times 100$$

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Gentamicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Gentamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Gentamicin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², without putting a filter paper in

the container, and air-dry the plate. Allow the plate to stand in iodine vapor, and compare the colored spots while covering with a glass plate: the spots other than the spots of gentamicin C_1 (*R*f value: about 0.3), gentamicin C_2 (*R*f value: about 0.2) and gentamicin C_{1a} (*R*f value: about 0.1) obtained from the sample solution are not more intense than the spot of gentamicin C_2 obtained from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 18.0% (0.15 g, reduced pressure not exceeding 0.67 kPa, 110°C, 3 hours). Handle the sample avoiding absorption of moisture.

Residue on ignition ⟨*2.44*⟩ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Staphylococcus epidermidis* ATCC 12228

(ii) Agar media for seed and base layer—

Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

(iii) Agar medium for transferring test organisms—Use the medium ii in 2) Medium for other organisms under (2) Agar media for transferring test organisms.

(iv) Standard solutions—Weigh accurately an amount of Gentamicin Sulfate RS, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 15°C or lower, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(v) Sample solutions—Weigh accurately an amount of Gentamicin Sulfate, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Gentamicin Sulfate Ophthalmic Solution

ゲンタマイシン硫酸塩点眼液

Gentamicin Sulfate Ophthalmic Solution is an aqueous ophthalmic preparation.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of expressed as mass of gentamicin C_1 ($C_{21}H_{43}N_5O_7$: 477.60).

Method of preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Gentamicin Sulfate.

Description Gentamicin Sulfate Ophthalmic Solution is a clear, colorless or pale yellow liquid.

Identification To a volume of Gentamicin Sulfate Ophthalmic Solution, equivalent to 10 mg (potency) of Gentamicin Sulfate, add water to make 5 mL, and use this solution as the sample solution. Separately, dissolve an amount of Gentamicin Sulfate RS, equivalent to 10 mg (potency), in 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the lower layer of a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat the plate at 100°C for 5 minutes: three principal spots obtained from the sample solution are the same with the corresponding spots obtained from the standard solution in color tone and the *R*f value, respectively.

pH ⟨*2.54*⟩ 5.5 – 7.5

Foreign insoluble matter ⟨*6.11*⟩ It meets the requirement.

Insoluble particulate matter ⟨*6.08*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, agar media for seed and base layer, agar medium for transferring test organism, and standard solutions—Proceed as directed in the Assay under Gentamicin Sulfate.

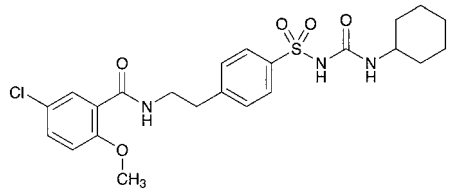
(ii) Sample solutions—Pipet a volume of Gentamicin Sulfate Ophthalmic Solution, equivalent to about 12 mg (potency) of Gentamicin Sulfate, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make a solution so that each mL contains about 1 mg (potency). Pipet a suitable volume of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Shelf life 24 months after preparation.

Glibenclamide

グリベンクラミド



$C_{23}H_{28}ClN_3O_5S$: 494.00
4-[2-(5-Chloro-2-methoxybenzoylamino)ethyl]-*N*-(cyclohexylcarbamoyl)benzenesulfonamide
[*10238-21-8*]

Glibenclamide, when dried, contains not less than 98.5% of glibenclamide ($C_{23}H_{28}ClN_3O_5S$).

Description Glibenclamide occurs as white to pale yellowish white, crystals or crystalline powder.

It is freely soluble in dimethylformamide, sparingly soluble in chloroform, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Glinbenclamide in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Glibenclamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Glibenclamide as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Melting point ⟨*2.60*⟩ 169 – 174°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Glibenclamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.20 g of Glibenclamide in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, chloroform and diluted ammonia TS (4 in 5) (11:7:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

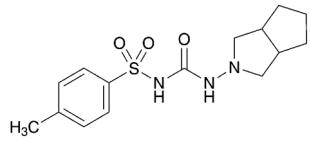
Assay Weigh accurately about 0.9 g of Glibenclamide, previously dried, dissolve in 50 mL of *N*,*N*-dimethylformamide, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination with a solution prepared by adding 18 mL of water to 50 mL of *N*,*N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 49.40 mg of $C_{23}H_{28}ClN_3O_5S$

Containers and storage Containers—Tight containers.

Gliclazide

グリクラジド



$C_{15}H_{21}N_3O_3S$: 323.41
1-(Hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methylphenyl)sulfonyl]urea
[*21187-98-4*]

Gliclazide, when dried, contains not less than 98.5% and not more than 101.0% of gliclazide ($C_{15}H_{21}N_3O_3S$).

Description Gliclazide is a white crystalline powder.

It is sparingly soluble in acetonitrile and in methanol, slightly soluble in ethanol (99.5) and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Gliclazide in methanol (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Gliclazide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 165 – 169°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Gliclazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure within 2 hours after preparation of the sample solution. Dissolve 50 mg of Gliclazide in 23 mL of acetonitrile, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water and acetonitrile (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than gliclazide obtained from the sample solution is not larger than the peak area of gliclazide obtained from the standard solution, and the total area of the peaks other than

the peak of gliclazide from the sample solution is not larger than 3 times the peak area of gliclazide from the standard solution. For the area of the peak, having the relative retention time of about 0.9 to gliclazide, multiply the relative response factor 5.65.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile, triethylamine and trifluoroacetic acid (550:450:1:1).

Flow rate: Adjust so that the retention time of gliclazide is about 14 minutes.

Time span of measurement: About 2 times as long as the retention time of gliclazide, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add a mixture of water and acetonitrile (11:9) to make exactly 20 mL. Confirm that the peak area of gliclazide obtained from 20 μL of this solution is equivalent to 10 to 30% of that of gliclazide obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of gliclazide are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gliclazide is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

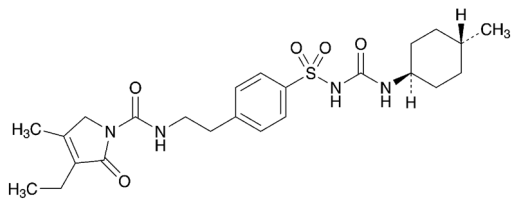
Assay Weigh accurately about 0.3 g of Gliclazide, previously dried, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.34 mg of $C_{15}H_{21}N_3O_3S$

Containers and storage Containers—Well-closed containers.

Glimepiride

グリメピリド



$C_{24}H_{34}N_4O_5S$: 490.62

1-(4-{2-[(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carbonyl)amino]ethyl}phenylsulfonyl)-3-(*trans*-4-methylcyclohexyl)urea

[*93479-97-1*]

Glimepiride contains not less than 98.0% and not more than 102.0% of glimepiride ($C_{24}H_{34}N_4O_5S$), calculated on the anhydrous basis.

Description Glimepiride occurs as a white crystalline powder.

It is slightly soluble in dichloromethane, very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 202°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Glimepiride in methanol (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Glimepiride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Glimepiride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Glimepiride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Glimepiride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) *cis*-Isomer—Dissolve 10 mg of Glimepiride in 5 mL of dichloromethane, add the mobile phase to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 to glimepiride, obtained from the sample solution is not larger than 3/4 times the peak area of glimepiride obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 3 mm in inside diameter and 15 cm in length, packed with diol silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of heptane for liquid chromatography, 2-propanol for liquid chromatography, and acetic acid (100) (900:100:1).

Flow rate: Adjust so that the retention time of glimepiride is about 14 minutes.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of glimepiride obtained with 10 μL of this solution is equivalent to 35 to 65% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 2.0%.

(3) Related substances—Keep the sample solution and standard solution below 4°C after preparing. Dissolve 20 mg of Glimepiride in 100 mL of a mixture of acetonitrile for liquid chromatography and water (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.25 to glimepiride, obtained from the sample solution is not larger than 4 times the peak area of glimepiride obtained from the standard solution, the area of the peak, having the relative retention time of about 1.1, is not larger than 2 times the peak area of glimepiride from the standard solution, the area of the peak, having the relative retention time of about 0.32, is not larger than 1.5 times the peak area of glimepiride from the standard solution, the area of peak other than glimepiride and above mentioned peak from the sample solution is not larger than the peak of glimepiride from the standard solution, and the total area of the peaks other than glimepiride and the peak, having the relative retention time of about 0.25 to glimepiride, from the sample solution is not larger than 5 times the peak area of glimepiride from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of glimepiride, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of glimepiride obtained with 20 μL of this solution is equivalent to 35 to 65% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 9000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 2.0%.

Water <*2.48*> Not more than 0.5% (0.25 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 20 mg each of Glimepiride and Glimepiride RS (separately determine the water <*2.48*> in the same manner as Glimepiride), dissolve each substance in a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glimepiride in each solution.

$$\text{Amount (mg) of glimepiride } (C_{24}H_{34}N_4O_5S) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glimepiride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.5 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, adjust to pH 2.5 with phosphoric acid, and add 500 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of glimepiride is about 17 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 9000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Glimepiride Tablets

グリメピリド錠

Glimepiride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$: 490.62).

Method of preparation Prepare as directed under Tablets, with Glimepiride.

Identification To a quantity of powdered Glimepiride Tablets, equivalent to 20 mg of Glimepiride, add 40 mL of acetonitrile, shake for 15 minutes, and centrifuge. Evaporate the supernatant liquid on a water bath under reduced pres-

sure, suspend the residue with 1 mL of water, and filter under reduced pressure. Wash the residue with 1 mL of water, dry at 105°C for 1 hour. Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3370 cm^{-1}, 3290 cm^{-1}, 2930 cm^{-1}, 1708 cm^{-1}, 1674 cm^{-1}, 1347 cm^{-1}, 1156 cm^{-1} and 618 cm^{-1}.

Purity Related substances—Keep the sample solution and standard solution below 4°C after preparation. To a quantity of powdered Glimepiride Tablets, equivalent to 9 mg of Glimepiride, wet with 0.5 mL of water, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 50 mL, shake, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 to glimepiride, obtained from the sample solution is not larger than 2.6 times the peak area of glimepiride obtained from the standard solution, the area of the peak other than glimepiride and the peak mentioned above from the sample solution is not larger than 3/10 times the peak area of glimepiride from the standard solution, and the total area of the peaks other than glimepiride and the peak mentioned above from the sample solution is not larger than the peak area of glimepiride from the standard solution, and the total area of the peaks other than glimepiride from the sample solution is not larger than 3 times the peak area of glimepiride from the standard solution.

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Flow rate: Adjust so that the retention time of glimepiride is about 12 minutes.

Time span of measurement: About 2 times as long as the retention time of glimepiride.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of glimepiride obtained with 5 μL of this solution is equivalent to 7 to 13% of that obtained with 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glimepiride is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Glimepiride Tablets add $V/10$ mL of water, disintegrate, add $V/2$ mL of a mixture of acetonitrile for liquid chromatography and water (4:1), and shake. To this solution add exactly $V/5$ mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly V mL so that each mL contains about 100 μg of glimepiride ($C_{24}H_{34}N_4O_5S$), and centrifuge. To 2.5 mL of the supernatant liquid add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glimepiride RS (separately determine the water <2.48> in the same manner as Glimepiride), and dissolve in a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of glimepiride } (C_{24}H_{34}N_4O_5S) = M_S \times Q_T/Q_S \times V/200$$

M_S: Amount (mg) of Glimepiride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile for liquid chromatography and water (4:1) (1 in 1000).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution (pH 7.5) as the dissolution medium, the dissolution rate in 15 minutes of 0.5-mg and 1-mg tablets is not less than 75%, and that in 30 minutes of 3-mg tablet is not less than 70%.

Start the test with 1 tablet of Glimepiride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 0.56 μg of glimepiride ($C_{24}H_{34}N_4O_5S$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Glimepiride RS (separately determine the water <2.48> in the same manner as Glimepiride), and dissolve in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 2 mL of this solution, add 8 mL of acetonitrile for liquid chromatography, and add the dissolution medium to make exactly 200 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glimepiride in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of glimepiride } (C_{24}H_{34}N_4O_5S) = M_S \times A_T/A_S \times V'/V \times 1/C \times 9/4$$

M_S: Amount (mg) of Glimepiride RS taken, calculated on the anhydrous basis

C: Labeled amount (mg) of glimepiride ($C_{24}H_{34}N_4O_5S$) in 1 tablet

Operating conditions—

Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glimepiride are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operations conditions, the relative standard deviation of the peak area of glimepiride is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Glimepiride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of glimepiride ($C_{24}H_{34}N_4O_5S$), add 3 mL of water, and shake with 30 mL of a mixture of acetonitrile for liquid chromatography and water (4:1). Add exactly 6 mL of the internal standard solution, and add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Glimepiride RS, (separately, determine the water <*2.48*> in the same manner as Glimepiride), dissolve in a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL. Pipet 15 mL of this solution, add exactly 6 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of glimepiride to that of the internal standard.

$$\text{Amount (mg) of glimepiride } (C_{24}H_{34}N_4O_5S) = M_S \times Q_T/Q_S \times 3/20$$

M_S: Amount (mg) of Glimepiride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile for liquid chromatography and water (4:1) (1 in 1000).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 228 nm).

Column: A stainless steel column 4 mm in inside diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.5 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, add 500 mL of acetonitrile for liquid chromatography, and adjust to pH 3.5 with diluted phosphoric acid (1 in 5).

Flow rate: Adjust so that the retention time of glimepiride is about 10 minutes.

System suitability—

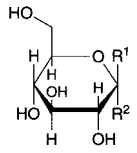
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and glimepiride are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of glimepiride to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Glucose

ブドウ糖



α-D-Glucopyranose : R^1=H, R^2=OH
β-D-Glucopyranose : R^1=OH, R^2=H

$C_6H_{12}O_6$: 180.16
D-Glucopyranose
[*50-99-7*]

Glucose is α-D-glucopyranose, β-D-glucopyranose, or a mixture of them.

It, when dried, contains not less than 99.5% of glucose [D-glucopyranose ($C_6H_{12}O_6$)].

Description Glucose occurs as white, crystals or crystalline powder. It is odorless, and has a sweet taste.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification Add 2 to 3 drops of a solution of Glucose (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.

Purity **(1)** Clarity and color of solution—Add 25 g of Glucose to 30 mL of water in a Nessler tube, warm at 60°C in a water bath until solution is effected, cool, and add water to make 50 mL: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS, and 2.0 mL of Copper (II) Sulfate CS, add water to make 10.0 mL. To 3.0 mL of this solution add water to make 50 mL.

(2) Acidity—Dissolve 5.0 g of Glucose in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS and 0.60 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <*1.03*>—Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(4) Sulfate <*1.14*>—Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Heavy metals <*1.07*>—Proceed with 5.0 g of Glucose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(6) Arsenic <*1.11*>—Dissolve 1.5 g of Glucose in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, and concentrate to 5 mL. After cooling, perform the test with this solution as the test solution (not more than 1.3 ppm).

(7) Dextrin—To 1.0 g of Glucose add 20 mL of ethanol (95), and boil under a reflux condenser: the solution is clear.

(8) Soluble starch and sulfite—Dissolve 1.0 g of Glucose in 10 mL of water, and add 1 drop of iodine TS: a yellow color develops.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C,

6 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (2 g).

Assay Weigh accurately about 10 g of Glucose, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, allow to stand for 30 minutes, and determine the optical rotation, α_D, of this solution at 20 ± 1°C in a 100-mm cell as directed under Optical Rotation Determination ⟨*2.49*⟩.

Amount (mg) of glucose ($C_6H_{12}O_6$) = α_D × 1895.4

Containers and storage Containers—Tight containers.

Glucose Injection

ブドウ糖注射液

Glucose Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of glucose ($C_6H_{12}O_6$: 180.16).

Method of preparation Prepare as directed under Injections, with Glucose. No preservative is added.

Description Glucose Injection is a clear, colorless liquid. It has a sweet taste. It occurs as a colorless to pale yellow, clear liquid when its labeled concentration exceeds 40%.

Identification Measure a volume of Glucose Injection, equivalent to 0.1 g of Glucose, and, if necessary, add water or evaporate on a water bath to a volume of 2 mL. Add 2 to 3 drops of the solution to 5 mL of boiling Fehling's TS: a red precipitate is produced.

pH ⟨*2.54*⟩ 3.5 – 6.5 In the case where the labeled concentration of the injection exceeds 5%, dilute to 5% with water before the test.

Purity 5-Hydroxymethylfurfural and related substances—Measure exactly a volume of Glucose Injection, equivalent to 2.5 g of Glucose, and add water to make exactly 100 mL. Determine the absorbance of this solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it is not more than 0.80.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

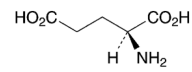
Assay Measure accurately a volume of Glucose Injection, equivalent to about 4 g of glucose ($C_6H_{12}O_6$), and add 0.2 mL of ammonia TS and water to make exactly 100 mL. Shake the solution well, allow to stand for 30 minutes, and determine the optical rotation, α_D, at 20 ± 1°C in a 100-mm cell as directed under Optical Rotation Determination ⟨*2.49*⟩.

Amount (mg) of glucose ($C_6H_{12}O_6$) = α_D × 1895.4

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

L-Glutamic Acid

L-グルタミン酸



$C_5H_9NO_4$: 147.13
(2*S*)-2-Aminopentanedioic acid
[*56-86-0*]

L-Glutamic Acid contains not less than 99.0% and not more than 101.0% of L-glutamic acid ($C_5H_9NO_4$), calculated on the dried basis.

Description L-Glutamic acid occurs as white, crystals or crystalline powder. It has a slight characteristic and acid taste.

It is slightly soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 2 mol/L hydrochloric acid TS.

It shows crystal polymorphism.

Identification Determine the infrared absorption spectrum of L-Glutamic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve L-Glutamic Acid in a small amount of water, evaporate water at 60°C under reduced pressure, and perform the test in the same manner with the dried residue.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +31.5 – +32.5° (2.5 g calculated on the dried basis, 2 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 0.7 g of L-Glutamic Acid in 100 mL of water by warming and then cooling is 2.9 to 3.9.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Glutamic Acid in 10 mL of 2 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of L-Glutamic Acid in 6 mL of dilute nitric acid and 20 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.6 g of L-Glutamic Acid in 5 mL of dilute hydrochloric acid and 30 mL of water, and add water to make 45 mL. Perform the test using this solution as the test solution. Prepare the control solution from 0.35 mL of 0.005 mol/L sulfuric acid VS and 5 mL of dilute hydrochloric acid, and dilute with water to 45 mL. Prepare the test solution and the control solution with 5 mL of barium chloride TS, respectively (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Glutamic Acid. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of L-Glutamic Acid in 20 mL of water and 7 mL of a solution of sodium hydroxide (1 in 25) by warming, cool, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution from 1.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not

more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Glutamic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of L-Glutamic Acid, and dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount, equivalent to 2.5 mmol, of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-arginine, dissolve them in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 6 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the mass percentage of each amino acid, using the mass of amino acid other than glutamic acid in 1 mL of the sample solution obtained from the height of the peaks obtained from the sample solution and standard solution: the amount of each amino acid other than glutamic acid is not more than 0.2%, and the total amount of these amino acids is not more than 0.6%.

Operating conditions—

Detector: A visible absorption photometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Reaction time: About 1 minute.

Mobile phase: Prepare the mobile phases A, B, C, D and E according to the following table, and add 0.1 mL each of caprylic acid.

Mobile phase	A	B	C	D	E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	—
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	—
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	—
Sodium hydroxide	—	—	—	—	8.00 g
Ethanol (99.5)	130 mL	20 mL	4 mL	—	100 mL
Thiodiglycol	5 mL	5 mL	5 mL	—	—
Benzyl alcohol	—	—	—	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL	4 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Changing of mobile phase: Switch the mobile phases A, B, C, D and E sequentially so that when proceed with 20 μL of the standard solution under the above conditions, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagent: Dissolve 204 g of lithium acetate dihydrate in an appropriate amount of water, add 123 mL of acetic acid (100), 401 mL of 1-methoxy-2-propanol and water to make 1000 mL, introduce nitrogen for 10 minutes, and use this solution as Solution (I). Separately, to 979 mL of 1-methoxy-2-propanol add 39 g of ninhydrin, introduce nitrogen for 5 minutes, add 81 mg of sodium borohydride, introduce nitrogen for 30 minutes, and use this solution as Solution (II). Prepare a mixture with an equal volume of the Solution (I) and Solution (II) (Prepare before use).

Flow rate of mobile phase: 0.20 mL per minute.

Flow rate of reaction regent: 0.24 mL per minute.

System suitability—

System performance: When the test is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and L-alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of each amino acid in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time of them is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

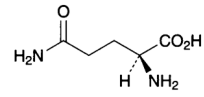
Assay Weigh accurately about 0.12 g of L-Glutamic Acid, dissolve in 40 mL of water by warming, cool, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 14.71 mg of $C_5H_9NO_4$

Containers and storage Containers—Tight containers.

L-Glutamine

L-グルタミン



$C_5H_{10}N_2O_3$: 146.14
(2*S*)-2,5-Diamino-5-oxopentanoic acid
[*56-85-9*]

L-Glutamine, when dried, contains not less than 99.0% and not more than 101.0% of L-glutamine ($C_5H_{10}N_2O_3$).

Description L-Glutamine occurs as white, crystals or a crystalline powder. It has a slight characteristic taste.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (99.5).

Identification Determine the infrared absorption spectrum of L-Glutamine as directed in the potassium bromide disk

method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +6.3 – +7.3° Weigh accurately about 2 g of L-Glutamine, previously dried, add 45 mL of water, warm to 40°C to dissolve, and after cooling, add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100-mm cell, within 60 minutes.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of L-Glutamine in 50 mL of water is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.5 g of L-Glutamine in 20 mL of water is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Glutamine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Glutamine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.10 g of L-Glutamine, using the distillation under reduced pressure. Prepare the control solution with 10.0 mL of Standard Ammonium Solution. The temperature of the water bath is 45°C (not more than 0.1%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Glutamine according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of L-Glutamine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Glutamine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on Ignition <2.44> Not more than 0.1% (1 g).

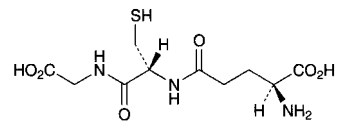
Assay Weigh accurately about 0.15 g of L-Glutamine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.61 mg of $C_5H_{10}N_2O_3$

Containers and storage Containers—Tight containers.

Glutathione

グルタチオン



$C_{10}H_{17}N_3O_6S$: 307.32
(2*S*)-2-Amino-4-[1-(carboxymethyl)carbamoyl-(2*R*)-2-sulfanylethylcarbamoyl]butanoic acid
[*70-18-8*]

Glutathione, when dried, contains not less than 98.0% and not more than 101.0% of glutathione ($C_{10}H_{17}N_3O_6S$).

Description Glutathione occurs as a white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Melting point: about 185°C (with decomposition).

Identification Determine the infrared absorption spectrum of Glutathione, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: −15.5 – −17.5° (after drying, 2 g, water, 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Glutathione in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Glutathione according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Glutathione according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Glutathione in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 4 to glutathione obtained from sample solution is not larger than 3/4 times the peak area of glutathione obtained from the standard solution, and the total area of the peaks other than glutathione is not larger than the peak area of glutathione from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen

phosphate and 2.02 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 970 mL of this solution add 30 mL of methanol.

Flow rate: Adjust so that the retention time of glutathione is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of glutathione, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of glutathione obtained from 10 μL of this solution is equivalent to 8 to 12% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 50 mg of glutathione, 10 mg of D-phenylglycine and 50 mg of ascorbic acid in 100 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, ascorbic acid, glutathione and D-phenylglycine are eluted in this order, and the resolutions between the peaks of ascorbic acid and glutathione and between the peaks of glutathione and D-phenylglycine are not less than 5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glutathione is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Glutathione, previously dried, dissolve in 50 mL of a solution of metaphosphoric acid (1 in 50), and titrate ⟨*2.50*⟩ with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 30.73 mg of $C_{10}H_{17}N_3O_6S$

Containers and storage Containers—Tight containers.

Glycerin

Glycerol

グリセリン

$C_3H_8O_3$: 92.09

Glycerin contains not less than 84.0% and not more than 87.0% of glycerin ($C_3H_8O_3$).

Description Glycerin is a clear, colorless, viscous liquid. It has a sweet taste.

It is miscible with water and with ethanol (99.5).

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Glycerin as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.449 – 1.454

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.221 – 1.230

Purity **(1)** Color—Place 50 mL of Glycerin in a Nessler tube, and observe downward: the solution has no more color than the following control solution.

Control solution: Place 0.40 mL of Iron (III) Chloride CS in a Nessler tube, and add water to make 50 mL.

(2) Acidity or alkalinity—To 2 mL of Glycerin add 8 mL of water and mix: the solution is neutral.

(3) Chloride ⟨*1.03*⟩—Take 10.0 g of Glycerin, and perform the test: Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001%).

(4) Sulfate ⟨*1.14*⟩—Take 10.0 g of Glycerin, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(5) Ammonium—To 5 mL of Glycerin add 5 mL of a solution of sodium hydroxide (1 in 10), and boil: the gas evolved does not change moistened red litmus paper to blue.

(6) Heavy metals ⟨*1.07*⟩—Proceed with 5.0 g of Glycerin according to Method 1, and perform the test: Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(7) Calcium—To 5 mL of the solution obtained in (2) add 3 drops of ammonium oxalate TS: the solution remains unchanged.

(8) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Glycerin according to Method 1, and perform the test (not more than 2 ppm).

(9) Acrolein, glucose, and other reducing substances—To 1.0 g of Glycerin add 1 mL of ammonia TS, mix, and warm in a water bath at 60°C for 5 minutes: no yellow color is produced. Take the solution out of the water bath, add 3 drops of silver nitrate TS immediately, and allow to stand in a dark place for 5 minutes: the color of the solution does not change, and no turbidity is produced.

(10) Fatty acids and esters—Mix 50 g of Glycerin with 50 mL of freshly boiled and cooled water, add exactly 10 mL of 0.1 mol/L sodium hydroxide VS, boil the mixture for 15 minutes, cool, and titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS: 0.1 mol/L sodium hydroxide VS consumed is not more than 3.0 mL (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

(11) Ethylene glycol, diethylene glycol and related substances—Weigh accurately about 5.88 g of Glycerin, mix with methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g each of ethylene glycol and diethylene glycol, mix with methanol to make exactly 100 mL. Pipet 5 mL of this solution and transfer into a 100-mL volumetric flask. Separately, weigh 5.0 g of glycerin for gas chromatography, mix with a suitable amount of methanol and put in the volumetric flask, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine the peak areas, A_{T1} and A_{S1}, of ethylene glycol and, A_{T2} and A_{S2}, of diethylene glycol by the automatic integration method. The amounts of ethylene glycol and diethylene glycol, calculated by the following equations, are not more than 0.1%, respectively. The amount of the peak other than glycerin, ethylene glycol and diethylene glycol obtained from the sample solution, calculated by the area percentage method, is not more than 0.1%, and the total amount of the peaks other than glycerin is not more than 1.0%.

Amount (%) of ethylene glycol
$= M_{S1}/M_T \times A_{T1}/A_{S1} \times 5$

Amount (%) of diethylene glycol
$= M_{S2}/M_T \times A_{T2}/A_{S2} \times 5$

M_{S1}: Amount (g) of ethylene glycol taken
M_{S2}: Amount (g) of diethylene glycol taken
M_T: Amount (g) of Glycerin taken

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column 0.32 mm in inside diameter and 30 m in length, coated the inner surface with 14% cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography 1 μm in thickness.

Column temperature: Inject at a constant temperature of about 100°C, raise the temperature at the rate of 7.5°C per minute to 220°C, and maintain at a constant temperature of about 220°C.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: about 38 cm per second.

Split ratio: 1:20.

Time span of measurement: About 3 times as long as the retention time of glycerin, beginning after the solvent peak.

System suitability—

System performance: Mix 50 mg each of ethylene glycol, diethylene glycol and glycerin for gas chromatography with 100 mL of methanol. When the procedure is run with 1 μL of this solution under the above operating conditions, ethylene glycol, diethylene glycol and glycerin are eluted in this order, and the resolution between the peaks of ethylene glycol and diethylene glycol is not less than 40, and between the peaks of diethylene glycol and glycerin is not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of ethylene glycol and diethylene glycol are not more than 10%, respectively.

(12) Readily carbonizable substances—To 5 mL of Glycerin add carefully 5 mL of sulfuric acid for readily carbonizable substances, mix gently at a temperature between 18°C and 20°C, and allow to stand for 1 hour between 15°C and 25°C: the solution has not more color than Matching Fluid H.

Water ⟨*2.48*⟩ 13 – 17% (0.1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Weigh accurately about 10 g of Glycerin in a tared crucible, heat to boiling, and fire to burn immediately. After cooling, moisten the residue with 1 to 2 drops of sulfuric acid, and ignite cautiously to constant mass: the mass of the residue is not more than 0.01%.

Assay Weigh accurately about 0.2 g of Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at a room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

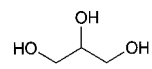
Each mL of 0.1 mol/L sodium hydroxide VS
$= 9.209$ mg of $C_3H_8O_3$

Containers and storage Containers—Tight containers.

Concentrated Glycerin

Concentrated Glycerol

濃グリセリン



$C_3H_8O_3$: 92.09
Propane-1,2,3-triol
[*56-81-5*]

Concentrated Glycerin contains not less than 98.0% and not more than 101.0% of glycerin ($C_3H_8O_3$), calculated of the anhydrous basis.

Description Concentrated Glycerin is a clear, colorless and viscous liquid. It has a sweet taste.

It is miscible with water and with ethanol (99.5).

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Concentrated Glycerin as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index ⟨*2.45*⟩ n_D^{20}: Not less than 1.470.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: Not less than 1.258.

Purity **(1)** Color—Place 50 mL of Concentrated Glycerin in a Nessler tube, and observe downward: the solution has no more color than the following control solution.

Control solution: Pipet 0.40 mL of Iron (III) Chloride CS into a Nessler tube, and add water to make 50 mL.

(2) Acidity or alkalinity—To 2 mL of Concentrated Glycerin add 8 mL of water and mix: the solution is neutral.

(3) Chloride ⟨*1.03*⟩—Take 10.0 g of Concentrated Glycerin, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001%).

(4) Sulfate ⟨*1.14*⟩—Take 10.0 g of Concentrated Glycerin, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(5) Ammonium—To 5 mL of Concentrated Glycerin add 5 mL of a solution of sodium hydroxide (1 in 10), and boil: the gas evolved does not change moistened red litmus paper to blue.

(6) Heavy metals ⟨*1.07*⟩—Proceed with 5.0 g of Concentrated Glycerin according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(7) Calcium—To 5 mL of the solution obtained in (2) add 3 drops of ammonium oxalate TS: the solution remains unchanged.

(8) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Concentrated Glycerin according to Method 1, and perform the test (not more than 2 ppm).

(9) Acrolein, glucose, or other reducing substances—To 1.0 g of Concentrated Glycerin add 1 mL of ammonia TS, mix, and warm in a water bath at 60°C for 5 minutes: no yellow color is produced. Take the solution out of the water bath, add 3 drops of silver nitrate TS immediately, and allow

to stand in a dark place for 5 minutes: the color of the solution does not change, and no turbidity is produced.

(10) Fatty acids and esters—Mix 50 g of Concentrated Glycerin with 50 mL of freshly boiled and cooled water, add 10 mL of 0.1 mol/L sodium hydroxide VS, accurately measured, boil the mixture for 15 minutes, cool, and titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS: not more than 3.0 mL of 0.1 mol/L sodium hydroxide VS is consumed (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

(11) Ethylene glycol, diethylene glycol and related substances—Weigh accurately about 5 g of Concentrated Glycerin, mix with methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g each of ethylene glycol and diethylene glycol, mix with methanol to make exactly 100 mL. Pipet 5 mL of this solution and transfer into a 100-mL volumetric flask. Separately, weigh 5.0 g of glycerin for gas chromatography, mix with a suitable amount of methanol and put in the volumetric flask, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine the peak areas, A_{T1} and A_{S1}, of ethylene glycol and, A_{T2} and A_{S2}, of diethylene glycol by the automatic integration method. The amounts of ethylene glycol and diethylene glycol, calculated by the following equations, are not more than 0.1%, respectively. The amount of the peak other than glycerin, ethylene glycol and diethylene glycol obtained from the sample solution, calculated by the area percentage method, is not more than 0.1%, and the total amount of the peaks other than glycerin is not more than 1.0%.

$$\text{Amount (\%) of ethylene glycol} = M_{S1}/M_T \times A_{T1}/A_{S1} \times 5$$

$$\text{Amount (\%) of diethylene glycol} = M_{S2}/M_T \times A_{T2}/A_{S2} \times 5$$

M_{S1}: Amount (g) of ethylene glycol taken
M_{S2}: Amount (g) of diethylene glycol taken
M_T: Amount (g) of Concentrated Glycerin taken

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column 0.32 mm in inside diameter and 30 m in length, coated the inner surface with 14% cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography 1 μm in thickness.

Column temperature: Inject at a constant temperature of about 100°C, raise the temperature at the rate of 7.5°C per minute to 220°C, and maintain at a constant temperature of about 220°C.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: about 38 cm per second.

Split ratio: 1:20.

Time span of measurement: About 3 times as long as the retention time of glycerin, beginning after the solvent peak.

System suitability—

System performance: Mix 50 mg each of ethylene glycol, diethylene glycol and glycerin for gas chromatography with 100 mL of methanol. When the procedure is run with 1 μL of this solution under the above operating conditions, ethylene glycol, diethylene glycol and glycerin are eluted in this order, and the resolution between the peaks of ethylene glycol and diethylene glycol is not less than 40, and between the peaks of diethylene glycol and glycerin is not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of ethylene glycol and diethylene glycol are not more than 10%, respectively.

(12) Readily carbonizable substances—To 5 mL of Concentrated Glycerin add carefully 5 mL of sulfuric acid for readily carbonizable substances, mix gently at a temperature between 18°C and 20°C, and allow to stand for 1 hour between 15°C and 25°C: the solution has no more color than Matching Fluid H.

Water ⟨*2.48*⟩ Not more than 2.0% (6 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Weigh accurately about 10 g of Concentrated Glycerin in a tared crucible, heat to boiling, and fire to burn immediately. Cool, moisten the residue with 1 to 2 drops of sulfuric acid, and ignite cautiously to constant mass: the mass of the residue is not more than 0.01%.

Assay Weigh accurately about 0.2 g of Concentrated Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at a room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.209 mg of $C_3H_8O_3$

Containers and storage Containers—Tight containers.

Glycerin and Potash Solution

グリセリンカリ液

Method of preparation

Potassium Hydroxide	3 g
Glycerin	200 mL
Ethanol	250 mL
Aromatic substance	a suitable quantity
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Dissolve Potassium Hydroxide in a portion of Water, Purified Water or Purified Water in Containers, add Glycerin, Ethanol, a suitable quantity of aromatic substance and another portion of Water, Purified Water or Purified Water in Containers to volume, and filter. Concentrated Glycerin may be used in place of Glycerin.

Description Glycerin and Potash Solution is a clear, colorless liquid, having an aromatic odor.

The pH of a solution of Glycerin and Potash Solution (1 in 5) is about 12.

Specific gravity d_{20}^{20}: about 1.02

Identification **(1)** A solution of Glycerin and Potash So-

lution (1 in 2) is alkaline (potassium hydroxide).

(2) Place 10 mL of a solution of Glycerin and Potash Solution (1 in 10) in a glass-stoppered test tube, add 2 mL of sodium hydroxide TS and 1 mL of copper (II) sulfate TS, and shake: a blue color is produced (glycerin).

(3) Glycerin and Potash Solution responds to the Qualitative Tests <*1.09*> for potassium salt.

Containers and storage Containers—Tight containers.

Glyceryl Monostearate

モノステアリン酸グリセリン

Glyceryl Monostearate is a mixture of α- and β-glyceryl monostearate and other fatty acid esters of glycerin.

Description Glyceryl Monostearate occurs as white to light yellow, waxy masses, thin flakes, or granules. It has a characteristic odor and taste.

It is very soluble in hot ethanol (95), soluble in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water and in ethanol (95).

It is slowly affected by light.

Identification (1) Heat 0.2 g of Glyceryl Monostearate with 0.5 g of potassium hydrogen sulfate until thoroughly charred: the irritative odor of acrolein is perceptible.

(2) Dissolve 0.1 g of Glyceryl Monostearate in 2 mL of ethanol (95) by warming, heat with 5 mL of dilute sulfuric acid in a water bath for 30 minutes, and cool: a white to yellow solid is produced. This separated solid dissolves when shaken with 3 mL of diethyl ether.

Melting point <*1.13*> Not below 55°C.

Acid value <*1.13*> Not more than 15.

Saponification value <*1.13*> 157 – 170

Iodine value <*1.13*> Not more than 3.0. Use chloroform instead of cyclohexane.

Purity <*1.13*> Acidity or alkalinity—To 1.0 g of Glyceryl Monostearate add 20 mL of boiling water, and cool with swirling: the solution is neutral.

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

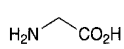
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Glycine

Aminoacetic Acid

グリシン



$C_2H_5NO_2$: 75.07
Aminoacetic acid
[*56-40-6*]

Glycine, when dried, contains not less than 98.5% of glycine ($C_2H_5NO_2$).

Description Glycine occurs as white, crystals or crystalline powder. It has a sweet taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

It shows crystal polymorphism.

Identification Determine the infrared absorption spectrum of Glycine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Glycine in water, evaporate the water to dryness, and repeat the test with the residue.

pH <*2.54*> Dissolve 1.0 g of Glycine in 20 mL of water: the pH of the solution is between 5.6 and 6.6.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Glycine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of Glycine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of Glycine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium<*1.02*>—Perform the test using 0.25 g of Glycine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of Glycine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Glycine according to Method 1, and perform the test (not more than 2 ppm).

(7) Related substences—Dissolve 0.10 g of Glycine in 25 mL of water and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

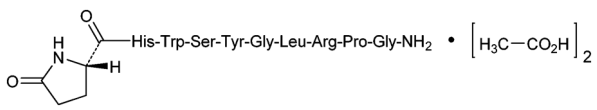
Assay Weigh accurately about 80 mg of Glycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 7.507 mg of $C_2H_5NO_2$

Containers and storage Containers—Well-closed containers.

Gonadorelin Acetate

ゴナドレリン酢酸塩



$C_{55}H_{75}N_{17}O_{13}.2C_2H_4O_2$: 1302.39
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-glycyl-L-leucyl-L-arginyl-L-prolyl-glycinamide diacetate
[*34973-08-5*]

Gonadorelin Acetate contains not less than 96.0% and not more than 102.0% of gonadorelin acetate ($C_{55}H_{75}N_{17}O_{13}.2C_2H_4O_2$), calculated on the anhydrous basis.

Description Gonadorelin Acetate occurs as a white to pale yellow powder. It is odorless or has a slight, acetic odor.

It is freely soluble in water, in methanol and in acetic acid (100), and sparingly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Gonadorelin Acetate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Gonadorelin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Gonadorelin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Gonadorelin Acetate in 0.5 mL of ethanol (99.5), add 1 mL of sulfuric acid, and heat: the odor of ethyl acetate is perceptible.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −53.0 – −57.0° (0.1 g calculated on the anhydrous basis, diluted acetic acid (100) (1 in 100), 10 mL, 100 mm).

pH <*2.54*> Dissolve 0.10 g of Gonadorelin Acetate in 10 mL of water: the pH of this solution is between 4.8 and 5.8.

Constituent amino acids Put 10 mg of Gonadorelin Acetate in a test tube for hydrolysis, add 0.5 mL of hydrochloric acid and 0.5 mL of a solution of mercaptoacetic acid (2 in 25), seal the tube under reduced pressure, and heat at 110°C for 5 hours. After cooling, open the tube, transfer the hydrolyzate into a beaker, and evaporate to dryness on a water bath. Add exactly 100 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh exactly 0.105 g of L-serine, 0.147 g of L-glutamic acid, 0.115 g of L-proline, 75 mg of glycine, 0.131 g of L-leucine, 0.181 g of L-tyrosine, 0.210 g of L-histidine hydrochloride monohydrate, 0.204 g of L-tryptophan and 0.211 g of L-arginine hydrochloride, which are all previously dried at 105°C for 3 hours, add 50 mL of 1 mol/L hydrochloric acid TS to dissolve them, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the peaks of nine constituent amino acids are observed on the chromatogram obtained with the sample solution, and their respective molar ratios to arginine are 0.7 – 1.0 for serine and tryptophan, 0.8 – 1.2 for proline, 0.9 – 1.1 for glutamic acid, leucine, tyrosine and histidine, respectively, and 1.8 – 2.2 for glycine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm for proline and 570 nm for others).

Column: A stainless steel column 4 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated polystyrene copolymer (5 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Mobile phase: Prepare the mobile phases A, B, C and D according to the following table.

Mobile phase	A	B	C	D
Trisodium citrate dihydrate	6.19 g	7.74 g	26.67 g	—
Sodium hydroxide	—	—	—	8.00 g
Sodium chloride	5.66 g	7.07 g	54.35 g	—
Citric acid monohydrate	19.80 g	22.00 g	6.10 g	—
Ethanol (99.5)	130 mL	20 mL	—	100 mL
Benzyl alcohol	—	—	5 mL	—
Thiodiglycol	5 mL	5 mL	—	—
Lauromacrogol solution in diethyl ether (1 in 4)	4 mL	4 mL	4 mL	4 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total volume	1000 mL	1000 mL	1000 mL	1000 mL

Flowing of mobile phase: Control the gradient by mixing the mobile phases A, B, C and D as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)
0 ~ 9	100	0	0	0
9 ~ 25	0	100	0	0
25 ~ 61	0	100 → 0	0 → 100	0
61 ~ 76	0	0	100	0
76 ~ 96	0	0	0	100

Reaction reagent: Dissolve 204 g of lithium acetate dihydrate in 336 mL of water, add 123 mL of acetic acid (100) and 401 mL of 1-methoxy-2-propanol, and use as Solution A. Separately, dissolve 39 g of ninhydrin and 81 mg of sodium borohydride in 979 mL of 1-methoxy-2-propanol, and use as Solution B. Mix the same volume of Solution A and Soluiton B before use.

Flow rate of mobile phase: 0.25 mL per minute.

Flow rate of reaction reagent: 0.3 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, serine, glutamic acid, proline, glycine, leucine, tyrosine, histidine, tryptophan and arginine are eluted in this order with enough separation between these peaks.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.10 g of Gonadorelin Acetate in 10 mL of water is clear, and the absorbance of this solution at 350

nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.10.

(2) Related substances—Dissolve 50 mg of Gonadorelin Acetate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than gonadorelin from the sample solution is not larger than 1/5 times the peak area of gonadorelin from the standard solution, and the total area of the peaks other than gonadorelin from the sample solution is not larger than 3/5 times the peak area of gonadorelin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of gonadorelin, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of gonadorelin obtained from 10 μL of this solution is equivalent to 1 to 3% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 4 mg of Gonadorelin Acetate in a suitable amount of the mobile phase, add 5 mL of a solution of phenacetin in acetonitrile (1 in 1000) and the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, gonadorelin and phenacetin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gonadorelin is not more than 5%.

Water <2.48> Not more than 8.0% (0.15 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (0.1 g).

Assay Weigh accurately about 20 mg of Gonadorelin Acetate and Gonadorelin Acetate RS (separately determine the water <2.48> in the same manner as Gonadorelin Acetate) and dissolve in diluted acetic acid (100) (1 in 1000) to make exactly 25 mL each. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and add water to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of gonadorelin to that of the internal standard.

$$\text{Amount (mg) of gonadorelin acetate } (C_{55}H_{75}N_{17}O_{13}.2C_2H_4O_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Gonadorelin Acetate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of phenacetin in a mixture of water and acetonitrile (3:2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0) and acetonitrile (90:17).

Flow rate: Adjust so that the retention time of gonadorelin is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, gonadorelin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of gonadorelin to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Human Chorionic Gonadotrophin

Chorionic Gonadotrophin

ヒト絨毛性性腺刺激ホルモン

Human Chorionic Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained from the urine of healthy pregnant women after the manufacturing process to remove or inactivate the virus.

It contains not less than 2500 human chorionic gonadotrophin Units per mg, and contains not less than 3000 chorionic gonadotrophin Units per mg protein.

It contains not less than 80% and not more than 125% of the labeled human chorionic gonadotrophin Units.

Description Human Chorionic Gonadotrophin occurs as a white to light yellow-brown powder.

It is freely soluble in water.

Identification Calculate b by the following equation, using Y_3 and Y_4 obtained in the Assay: b is not more than 120.

$$b = E/I$$

$$E = (Y_3 - Y_4)/f$$

f: Number of test animals per group

$$I = \log (T_H/T_L)$$

Purity **(1)** Clarity and color of solution—Dissolve 0.05 g of Human Chorionic Gonadotrophin in 5 mL of isotonic sodium chloride solution: the solution is clear and colorless or light yellow.

(2) Estrogen—Inject subcutaneously into each of three female albino rats or albino mice ovariectomized at least two weeks before the test, single dose of 100 units according to the labeled Units dissolved in 0.5 mL of isotonic sodium chloride solution. Take vaginal smear twice daily, on the third, fourth and fifth day. Place the smear thinly on a slide

glass, dry, stain with Giemsa's TS, wash with water, and again dry: no estrus figure is shown microscopically.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.03 EU/unit.

Abnormal toxicity Dilute Human Chorionic Gonadotrophin with isotonic sodium chloride solution so that each mL of the solution contains 120 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

Specific activity When calculate from the results obtained by the Assay and the following test, the specific activity is not less than 3000 human chorionic gonadotrophin Units per mg protein.

(i) Sample solution—To an exactly amount of Human Chorionic Gonadotrophin add water to make a solution so that each mL contains about 500 Units of human chorionic gonadotrophin.

(ii) Standard solution—Weigh accurately about 10 mg of bovine serum albumin, and dissolve in water to make exactly 20 mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50 μg of the albumin per mL, respectively.

(iii) Procedure—Pipet 0.5 mL each of the sample solution and standard solutions, put them in glass test tubes about 18 mm in inside diameter and about 130 mm in length, add exactly 5 mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30°C for 10 minutes. Then add exactly 0.5 mL of diluted Folin's TS (1 in 2), mix, and warm in a water bath at 30°C for 20 minutes. Determine the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a solution obtained in the same manner with 0.5 mL of water as the blank.

Plot the absorbances of the standard solutions on the vertical axis and their protein concentrations on the horizontal axis to prepare a calibration curve, and determine the protein content of the sample solution from its absorbance by using this curve. Then calculate the amount of the protein in the sample.

Assay (i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solution—Dissolve a quantity of Human Chorionic Gonadotrophin RS in bovine serum albumin-isotonic sodium chloride solution to prepare four kinds of solutions, having 7.5, 15, 30 and 60 Units per 2.5 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and weigh their ovaries, as directed in procedure of (iv). Inject bovine serum albumin-isotonic sodium chloride solution to another group, and use this group as the control group. According to the result of this test, designate the concentration of the reference standard which will increase the masses of the ovaries about 2.5 times the mass of the ovaries of the control group as a low-dose concentration of the standard solution, and the concentration 1.5 to 2.0 times the low-dose concentration as a high-dose concentration. Dissolve a quantity of Human Chorionic Gonadotrophin RS, in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose standard solution S_H and a low-dose standard solution S_L whose concentrations are equal to those determined by the above test.

(iii) Sample solution—According to the labeled units, weigh accurately a suitable quantity of Human Chorionic Gonadotrophin, dissolve in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose sample solution T_H and a low-dose sample solution T_L having Units equal to the standard solutions in equal volumes.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H, S_L, T_H and T_L in each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwonted tissues attached to the ovaries, and remove the adhering water by lightly pressing between filter paper, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

Units per mg of Human Chorionic Gonadotrophin
$= \text{antilog}\, M \times$ units per mL of $S_H \times b/a$

$M = IY_a/Y_b$
$I = \log(S_H/S_L) = \log(T_H/T_L)$
$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$
$Y_b = Y_1 - Y_2 + Y_3 - Y_4$

a: Mass (mg) of Human Chorionic Gonadotrophin taken
b: Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-isotonic sodium chloride solution

F' computed by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L (P = 0.95) by the following equation: L should be not more than 0.3. If F' exceeds F_1, or if L exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until F' is smaller than F_1 or L is not more than 0.3.

$$F' = (Y_1 - Y_2 - Y_3 + Y_4)^2/(4fs^2)$$

f: Number of test animals per group

$s^2 = \{\Sigma y^2 - (Y/f)\}/n$

Σy^2: The sum of the squares of each y_1, y_2, y_3 and y_4

$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$
$n = 4(f - 1)$

$$L = 2\sqrt{(C - 1)(CM^2 + I^2)}$$

$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$

t^2: Value shown in the following table against n used to calculate s^2

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Human Chorionic Gonadotrophin for Injection

Chorionic Gonadotrophin for Injection

注射用ヒト絨毛性性腺刺激ホルモン

Human Chorionic Gonadotrophin for Injection is a preparation for injection which is dissolved before use.

It contains not less than 80% and not more than 125% of the labeled human chorionic gonadotrophin Units.

Method of preparation Prepare as directed under Injections with Human Chorionic Gonadotrophin.

Description Human Chorionic Gonadotrophin for Injection occurs as a white to light yellow-brown powder or masses.

Identification Proceed as directed in the Identification under Human Chorionic Gonadotrophin.

pH ⟨*2.54*⟩ Prepare a solution so that each mL of isotonic sodium chloride solution contains 2 mg of Human Chorionic Gonadotorophin for Injection: the pH of this solution is between 5.0 and 7.0.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.03 EU/unit.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test, when calculate the acceptance value using the mean of estimated contents of the units tested as *M*.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Proceed as directed in the Assay under Human Chorionic Gonadotrophin. The ratio of the assayed Units to the labeled Units should be calculated by the following equation.

The ratio of the assayed Units to the labeled Units = antilog *M*

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, and in a cold place.

Human Menopausal Gonadotrophin

ヒト下垂体性性腺刺激ホルモン

Human Menopausal Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained from the urine of postmenopausal healthy women, after processing for virus removal or inactivation. It has follicle-stimulating hormonal action and luteinizing hormonal action.

It contains not less than 40 follicle-stimulating hormone Units per mg.

Description Human Menopausal Gonadotrophin occurs as a white to pale yellow powder.

It is soluble in water.

Purity Interstitial cell-stimulating hormone—Perform the test according to the following method: the ratio of the unit of interstitial cell-stimulating hormone (luteinizing hormone) to that of follicle-stimulating hormone is not more than 1. The luteinizing activity of the hormone is determined by the seminal vesicle weight assay or ovarian ascorbic acid depletion assay. The seminal vesicle weight assay may be used when the ratio of the unit of interstitial cell-stimulating hormone to that of follicle-stimulating hormone is not more than 1 and not less than 0.10.

1) Seminal vesicle weight assay

(i) Test animals—Select healthy male albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Dissolve Menopausal Gonadotrophin RS in bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) to prepare three kinds of solutions, containing 10, 20 and 40 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their seminal vesicles as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the seminal vesicle 20 to 35 mg, as the high-dose standard solution, S_H. Dilute the S_H to 1.5 to 2.0 times the initial volume with the bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) and designate this solution as the low-dose standard solution, S_L.

(iii) Sample solutions—Weigh accurately a suitable amount of Human Menopausal Gonadotrophin, and dissolve in the bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) to prepare the high-dose sample solution, T_H and the low-dose sample solution, T_L, so that their concentrations are similar to those of the corresponding standard solutions, respectively. Store these solutions at 2 – 8°C.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously once every day 0.2 mL each of S_H, S_L, T_H and T_L to each animal in the respective groups for five days. On the sixth day, excise the seminal vesicles, remove extraneous tissue, remove fluid adhering to the vesicles and the contents of the vesicles by lightly pressing between filter papers, and weigh the vesicles.

(v) Calculation—Proceed as directed in (v) in the Assay by changing the mass of ovaries to the mass of seminal vesicles to read.

2) Ovarian ascorbic acid depletion assay

(i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Dissolve Human Menopausal Gonadotrophin RS in bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) to prepare four kinds of solutions, containing 2, 4, 8 and 16 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and determine the amount of ovarian ascorbic acid. Separately, inject bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) to a

control group of animals. According to the result of the test, designate the concentration of the reference standard, which will make the amount of ovarian ascorbic acid 0.80 to 0.85 times that in the control group, as the concentration for the low-dose standard solution, and 4 to 6 times that as the concentration for the high-dose standard solution. Dissolve Human Menopausal Gonadotrophin RS in bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) to prepare the high-dose standard solution and low-dose standard solution to contain the concentrations described above, and designate them as S_H and S_L, respectively.

(iii) Sample solutions—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, and dissolve in the bovine serum albumin-sodium chloride-phosphate buffer solution (pH 7.2) to prepare the high-dose sample solution and low-dose sample solution to contain units equal to those of the high-dose standard solution and low-dose standard solution, and designate them as T_H and T_L, respectively.

(iv) Procedure—Inject subcutaneously to each animal 80 units of serum gonadotrophin dissolved in 0.5 mL of isotonic sodium chloride solution. At 56 to 72 hours after the injection, inject subcutaneously to each animal 40 units of human chorionic gonadotrophin dissolved in 0.5 mL of isotonic sodium chloride solution. On 6 to 9 days after the last injection, divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject 1 mL each of S_H, S_L, T_H and T_L into the tail vein of each animal in groups A, B, C and D, respectively. At 2 to 4 hours after the injection, excise the both ovaries, remove the fat and other unwanted tissues attached to the ovaries, weigh, add a prescribed volume between 5 and 15 mL of metaphosphoric acid solution (1 in 40), homogenize with a homogenizer on ice, and centrifuge. To 0.5 to 1 mL (1 mL in principle. 0.5 mL may be used when the absorbance is not more than 0.1) of the supernatant liquid, add 1.5 mL of metaphosphoric acid solution (1 in 40) and 2.5 mL of 2,6-dichloroindophenol sodium-sodium acetate TS, mix the solution, and immediately determine the absorbance of the solution at 520 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Separately, weigh accurately 10.0 mg of Ascorbic Acid RS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL. Pipet a suitable volume of this solution, and add metaphosphoric acid solution (1 in 40) to make a solution so that each mL contains 2.0 to 10.0 μg of ascorbic acid ($C_6H_8O_6$: 176.12). To 2.5 mL of this solution, add 2.5 mL of 2,6-dichloroindophenol sodium-sodium acetate TS, mix the solution, immediately determine the absorbance in the same manner as mentioned above, and prepare the calibration curve. From the calibration curve of ascorbic acid, determine the amount (mg) of ascorbic acid in 100 g of ovary.

(v) Calculation—Proceed as directed in (v) in the Assay by changing the mass of ovaries to the amount of ascorbic acid to read.

Bacterial endotoxins <*4.01*> Dissolve Human Menopausal Gonadotrophin in water for bacterial endotoxins test to prepare a solution containing 75 follicle-stimulating hormone Units per mL, and perform the test: less than 0.66 EU/ follicle-stimulating hormone Unit.

Water <*2.48*> Not more than 5.0% (0.2 g, volumetric titration, direct titration).

Specific activity Perform the test with Human Menopausal Gonadotrophin according to the following method, and calculate the specific activity using the amount (Unit) obtained in the Assay: it is not less than 50 follicle-stimulating hormone Units per 1 mg of protein.

(i) Sample solution—Weigh accurately about 10 mg of Human Menopausal Gonadotrophin, dissolve in water so that each mL contains exactly 200 μg, and use this solution as the sample solution.

(ii) Standard solutions—Weigh accurately about 10 mg of bovine serum albumin, and dissolve in water to make exactly 20 mL. To this solution add water to make four solutions containing exactly 300 μg, 200 μg, 100 μg and 50 μg of the albumin per mL, respectively, and use these solutions as the standard solutions.

(iii) Procedure—To glass test tubes, about 18 mm in inside diameter and about 130 mm in height, add separately exactly 0.5 mL each of the sample solution and the standard solutions. To these tubes add exactly 5 mL of alkaline copper TS, warm in a water bath at 30°C for 10 minutes, then add exactly 0.5 mL of diluted Folin's TS (1 in 2), and warm in a water bath at 30°C for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrometry <*2.24*>, and determine the absorbances at 750 nm, using a liquid obtained with 0.5 mL of water in the same manner as above as a blank.

Prepare a calibration curve from the absorbances of the standard solutions, with absorbance on the vertical axis and concentration on the horizontal axis. Calculate the amount of protein in the sample solution from the absorbance of the sample solution using the curve, and calculate the protein content of the sample.

Assay

(i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Dissolve Human Menopausal Gonadotrophin RS in human chorionic gonadotrophin TS to make three solutions which contain 0.75, 1.5 and 3.0 follicle-stimulating hormone Units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their ovaries, as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the ovary about 120 to 160 mg, as the high-dose standard solution, S_H. Dilute the S_H to 1.5 to 2.0 times the initial volume with the human chorionic gonadotrophin TS, and designate the solution as the low-dose standard solution, S_L.

(iii) Sample solutions—Weigh accurately a suitable amount of Human Menopausal Gonadotrophin, dissolve in human chorionic gonadotrophin TS, and prepare the high-dose sample solution, T_H, and the low-dose sample solution, T_L, which have similar numbers of units to those of corresponding standard solutions in equal volume, respectively.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.2 mL each of S_H, S_L, T_H and T_L into the animals in each group, once in the afternoon on the first day, three times in the morning, noon and afternoon on the second day, and two times in the morning and afternoon on the third day. On the fifth day, excise the ovaries, remove the fat and extraneous tissue, remove fluid adhering to the ovaries by lightly pressing between filter papers, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

$$\text{Units per mg of Human Menopausal Gonadotrophin} = \text{antilog } M \times (\text{units per mL of } S_H) \times b/a$$

$$M = IY_a/Y_b$$

$$I = \log(S_H/S_L) = \log(T_H/T_L)$$
$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$
$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a: Mass (mg) of Human Menopausal Gonadotrophin taken
b: Total volume (mL) of the high dose of the test solution prepared by diluting with human chorionic gonadotrophin TS

F' computed by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L (P = 0.95) by the following equation: L should be not more than 0.3. If F' exceeds F_1, or if L exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until F' is smaller than F_1 or L is not more than 0.3.

$$F' = (Y_1 - Y_2 - Y_3 + Y_4)^2/(4fs^2)$$

f: Number of test animals per group

$$s^2 = \{\Sigma y^2 - (Y/f)\}/n$$

Σy^2: The sum of the squares of each y_1, y_2, y_3 and y_4

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$
$$n = 4(f - 1)$$

$$L = 2\sqrt{(C - 1)(CM^2 + I^2)}$$

$$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$$

t^2: Value shown in the following table against n used to calculate s^2

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Gramicidin

グラミシジン

[*1405-97-6*]

Gramicidin is a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus brevis* Dubos.

It contains not less than 900 μg (potency) per mg, calculated on the dried basis. The potency of Gramicidin is expressed as mass (potency) of gramicidin.

Description Gramicidin occurs as a white to light yellowish white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water.

Identification **(1)** To 10 mg of Gramicidin add 2 mL of 6 mol/L hydrochloric acid TS, and heat in a water bath for 30 minutes with occasional stirring. After cooling, neutralize with 6 mol/L sodium hydroxide TS, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 2 minutes: a blue-purple to red-purple color develops.

(2) Determine the absorption spectrum of a solution of Gramicidin in ethanol (95) (1 in 20,000), as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Gramicidin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Loss on drying <*2.41*> Not more than 3.0% (0.1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 1.0% (1 g).

Assay Perform the test according to the Turbidimetric method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Enterococcus hirae* ATCC 10541

(ii) Agar medium for transferring test organism—

Glucose	10.0 g
Casein peptone	5.0 g
Yeast extract	20.0 g
Potassium dihydrogen phosphate	2.0 g
Polysorbate 80	0.1 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.7 to 6.8 after sterilization.

(iii) Liquid medium for suspending test organism—Use the culture medium (2).

(iv) Preparation of the test organism suspension—Puncture the test organism in the medium, prepared by dispensing 10 mL of the agar medium for transferring test organism in a test tube about 16 mm in inside diameter, incubate at 36.5 to 37.5°C for 20 to 24 hours. After sub-culturing at least three times, keep between 1 to 5°C. Transfer the organism so obtained in 10 mL of the liquid medium for suspending test organism, incubate at 36.5 to 37.5°C for 20 to 24 hours, and use this medium as the test organism stock suspension. Before use, add the test organism stock suspension to the liquid medium for suspending test organism so that the transmittance at 580 nm is 50 to 60%. Mix one volume of this suspension and 200 volume of the liquid medium for suspending test organism, and use this as the test organism suspension.

(v) Standard solution—Weigh accurately an amount of Gramicidin RS, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 3 hours, equivalent to about 10 mg (potency), dissolve in ethanol (99.5) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add the following diluting solution to make a solution so that each mL contains 0.02 μg (potency), and use this solution as the standard solution.

Diluting solution: To 390 mL of propylene glycol add 210 mL of a mixture of ethanol (99.5) and acetone (9:1) and Sterile Purified Water to make 1000 mL.

(vi) Sample solution—Weigh accurately an amount of Gramicidin, equivalent to about 10 mg (potency), and dis-

solve in ethanol (99.5) to make exactly 100 mL. Take exactly a suitable amount of this solution, add the diluting solution obtained in (v) to make a solution so that each mL contains 0.02 μg (potency), and use this solution as the sample solution.

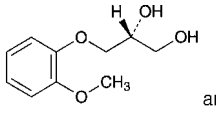
(vii) Procedure—Transfer 0.155 mL, 0.125 mL, 0.100 mL, 0.080 mL and 0.065 mL each of the standard solution, 0.100 mL of the sample solution and 0.100 mL of the diluting solution obtained in (v), separately, in test tubes about 14 mm in inside diameter and about 15 cm in length, and make three sets for each. To each of the test tube add 10 mL of the test organism suspension, stopper the tube, incubate in a water bath at 36.5 to 37.5°C for 180 to 270 minutes, add 0.5 mL of a solution of formaldehyde (1 in 3), and determine their transmittances at 580 nm.

Containers and storage Containers—Tight containers.

Guaifenesin

Guaiacol Glyceryl Ether

グアイフェネシン



and enantiomer

$C_{10}H_{14}O_4$: 198.22
(2*RS*)-3-(2-Methoxyphenoxy)propane-1,2-diol
[*93-14-1*]

Guaifenesin, when dried, contains not less then 98.0% and not more than 102.0% of guaifenesin ($C_{10}H_{14}O_4$).

Description Guaifenesin occurs as a white, crystals or crystalline powder.

It is freely soluble in ethanol (95), and sparingly soluble in water.

A solution of ethanol (95) (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Guaifenesin (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Guaifenesin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Guaifenesin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Guaifenesin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Dissolve 1.0 g of Guaifenesin in 100 mL of water: the pH of the solution is between 5.0 and 7.0.

Melting point <*2.60*> 80 – 83°C

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Guaifenesin in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.7 g of Guaifenesin in 25 mL of water by warming. Cool, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.020%).

(3) Heavy metals <*1.07*>—Dissolve 2.0 g of Guaifenesin in 25 mL of water by warming. Cool, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Guaifenesin according to Method 3, and perform the test (not more than 2 ppm).

(5) Free guaiacol—To 1.0 g of Guaifenesin add exactly 25 mL of water, dissolve by warming, cool, and use this solution as the sample solution. Separately, dissolve 0.100 g of guaiacol in water to make exactly 1000 mL. Pipet 3 mL of this solution, add exactly 22 mL of water, and use this solution as the standard solution. To each of the sample solution and standard solution add 1.0 mL of potassium hexacyanoferrate (III) TS and 5.0 mL of a solution of 4-aminoantipyrine (1 in 200), and immediately after shaking for exactly 5 seconds add a solution of sodium hydrogen carbonate (1 in 1200) to make exactly 100 mL. Determine the absorbances of these solutions at 500 nm exactly 15 minutes after the addition of the 4-aminoantipyrine solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared in the same manner with 25 mL of water, as the blank: the absorbance of the solution obtained from the sample solution is not more than that from the standard solution.

(6) Related substances—Dissolve 1.0 g of Guaifenesin in 100 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethanol (95), and ammonia solution (28) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg of Guaifenesin and Guaifenesin RS, previously dried, and dissolve each then in water to make exactly 100 mL. Pipet 5 mL of these solutions, and add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

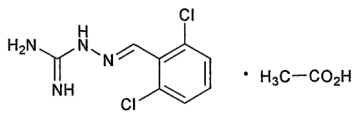
Amount (mg) of guaifenesin ($C_{10}H_{14}O_4$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Guaifenesin RS taken

Containers and storage Containers—Tight containers.

Guanabenz Acetate

グアナベンズ酢酸塩



$C_8H_8Cl_2N_4.C_2H_4O_2$: 291.13
(*E*)-1-(2,6-Dichlorobenzylideneamino)guanidine monoacetate
[*23256-50-0*]

Guanabenz Acetate, when dried, contains not less than 98.5% of guanabenz acetate ($C_8H_8Cl_2N_4.C_2H_4O_2$).

Description Guanabenz Acetate occurs as white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol and in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

It is gradually affected by light.

Melting point: about 190°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Guanabenz Acetate (1 in 1000) add 0.5 mL of a diluted ethanol (95) (5 in 6) which contains 16 g of urea and 0.2 g of 1-naphthol in 100 mL, and add 1 mL of *N*-bromosuccinimide TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of Guanabenz Acetate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Guanabenz Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 0.1 g of Guanabenz Acetate add 5 mL of water and 1 mL of ammonia TS, shake, filter, and neutralize the filtrate with dilute hydrochloric acid: the solution responds to the Qualitative Tests <*1.09*> (3) for acetate.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Guanabenz Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.05 g of Guanabenz Acetate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 10 mL, then pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (80:20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Place the plate in a chamber filled with iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

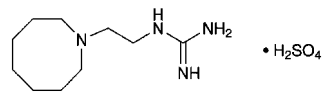
Assay Weigh accurately about 0.25 g of Guanabenz Acetate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.11 mg of $C_8H_8Cl_2N_4.C_2H_4O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Guanethidine Sulfate

グアネチジン硫酸塩



$C_{10}H_{22}N_4.H_2SO_4$: 296.39
1-[2-(Hexahydroazocin-1(2*H*)-yl)ethyl]guanidine monosulfate
[*645-43-2*]

Guanethidine Sulfate, when dried, contains not less than 98.5% of guanethidine sulfate ($C_{10}H_{22}N_4.H_2SO_4$).

Description Guanethidine Sulfate occurs as white, crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a bitter taste.

It is very soluble in formic acid, freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Melting point: 251 – 256°C (an evacuated sealed capillary tube, with decomposition).

Identification **(1)** To 4 mL of a solution of Guanethidine Sulfate (1 in 4000) add 2 mL of 1-naphthol TS, 1 mL of diacetyl TS and 15 mL of water, and allow to stand for 30 minutes: a red color develops.

(2) Determine the infrared absorption spectrum of Guanethidine Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Guanethidine Sulfate (1 in 10) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve 1.0 g of Guanethidine Sulfate in 50 mL of water: the pH of the solution is between 4.7 and 5.7.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Guanethidine Sulfate in 50 mL of water: the solution is clear and colorless.

(2) Methylisothiourea sulfate—Dissolve 2.0 g of Guanethidine Sulfate in 80 mL of sodium hydroxide TS, and allow to stand for 10 minutes. Add 60 mL of hydrochloric acid, 2 g of sodium bromide and water to make 200 mL. Then, to this solution add 0.70 mL of 1/60 mol/L potassium bromate VS and 2 mL of zinc iodide-starch paste TS: a blue color develops.

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Guanethidine Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Guanethidine Sulfate, previously dried, dissolve in 2 mL of formic acid, add 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.64 mg of $C_{10}H_{22}N_4.H_2SO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Freeze-dried Habu Antivenom, Equine

乾燥はぶウマ抗毒素

Freeze-dried Habu Antivenom, Equine, is a preparation for injection which is dissolved before use.

It contains *Trimeresurus flavoviridis* antivenom in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Habu Anti-venom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Habu Antivenom, Equine, becomes colorless or light yellow-brown, clear liquid or a slightly whitish turbid liquid on addition of solvent.

Adsorbed Habu-venom Toxoid

沈降はぶトキソイド

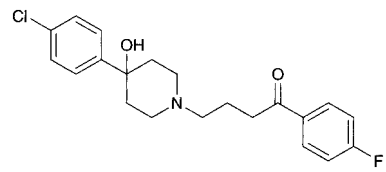
Adsorbed Habu-venom Toxoid is a liquid for injection containing habu toxoid prepared by treating toxic substances produced by habu (*Trimeresurus flavoviridis*) with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by the addition of aluminum salt.

It conforms to the requirements of Adsorbed Habu-venom Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Habu-venom Toxoid becomes a uniform whitish turbid liquid on shaking.

Haloperidol

ハロペリドール



$C_{21}H_{23}ClFNO_2$: 375.86
4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one
[*52-86-8*]

Haloperidol, when dried, contains not less than 99.0% and not more than 101.0% of haloperidol ($C_{21}H_{23}ClFNO_2$).

Description Haloperidol occurs as white to pale yellow, crystals or powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in 2-propanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Dissolve 30 mg of Haloperidol in 100 mL of 2-propanol. To 5 mL of the solution add 10 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Haloperidol as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 149 – 153°C

Purity **(1)** Sulfate <*1.14*>—To 1.0 g of Haloperidol add 50 mL of water, shake, and filter. To 25 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Haloperidol according to method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 25 mg of Haloperidol in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than haloperidol obtained from the sample solution is not larger than the peak area of haroperidol obtained from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of haloperidol from the standard solution. For the areas of the peaks, having the relative retention time of about 0.5, about 1.2 and about 2.6 to haloperidol, multiply their relative response factors, 0.75, 1.47 and 0.76, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of methanol and 1.0 g of sodium lauryl sulfate.

Flow rate: Adjust so that the retention time of haloperidol is about 9 minutes.

Time span of measurement: About 3 times as long as the retention time of haloperidol, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of haloperidol obtained with 10 μL of this solution is equivalent to 15 to 25% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of haloperidol are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloperidol is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 60°C, phosphorus (V) oxide, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of haloperidol, previously dried, and dissolve in 40 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 37.59 mg of $C_{21}H_{23}ClFNO_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Haloperidol Fine Granules

ハロペリドール細粒

Haloperidol Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$: 375.86).

Method of preparation Prepare as directed under Granules, with Haloperidol.

Identification Powder Haloperidol Fine Granules. To a portion of the powder, equivalent to 6 mg of Haloperidol, add 70 mL of 2-propanol, and heat to boiling on a water bath while shaking. After cooling, add 2-propanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 2 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 219 nm and 223 nm and between 243 nm and 247 nm.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Haloperidol Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Haloperidol Fine Granules, equivalent to about 3 mg of haloperidol ($C_{21}H_{23}ClFNO_2$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as a desiccant, and dissolve in methanol to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and determine the peak areas, A_T and A_S, of haloperidol in each solution.

Dissolution rate (%) with respect to the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 18$

M_S: Amount (mg) of haloperidol for assay taken
M_T: Amount (g) of Haloperidol Fine Granules taken
C: Labeled amount (mg) of haloperidol ($C_{21}H_{23}ClFNO_2$) in 1 g

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as detected in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of haloperidol are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloperidol is not more than 2.0%.

Assay Powder Haloperidol Fine Granules. Weigh accurately a portion of the powder, equivalent to about 10 mg of haloperidol ($C_{21}H_{23}ClFNO_2$), add 10 mL of water, disperse the particle with the aid of ultrasonic waves, add exactly 20 mL of the internal standard solution, extract for 30 minutes with the aid of ultrasonic waves with occasional shaking, and add the mobile phase to make 100 mL. Centrifuge after shaking for more 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following

conditions, and calculate the ratios, Q_T and Q_S, of the peak area of haloperidol to that of the internal standard.

$$\text{Amount (mg) of haloperidol } (C_{21}H_{23}ClFNO_2) = M_S \times Q_T/Q_S \times 2/5$$

M_S: Amount (mg) of haloperidol for assay taken

Internal standard solution—A solution of diphenyl in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 250 mL of this solution add 750 mL of methanol and 1.0 g of sodium lauryl sulfate, and mix to dissolve.

Flow rate: Adjust so that the retention time of haloperidol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Haloperidol Injection

ハロペリドール注射液

Haloperidol Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$: 375.86).

Method of preparation Prepare as directed under Injections, with Haloperidol.

Description Haloperidol Injection occurs as a colorless to pale yellow, clear liquid.

Identification To a volume of Haloperidol Injection, equivalent to 5 mg of Haloperidol, add 2-propanol to make 100 mL. To 5 mL of this solution add 2 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 219 nm and 223 nm and between 243 nm and 247 nm.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins ⟨*4.01*⟩ Less than 60 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Haloperidol Injection, equivalent to about 10 mg of haloperidol ($C_{21}H_{23}ClFNO_2$), add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C using phosphorus (V) oxide as a desiccant for 3 hours, dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of haloperidol in each solution.

$$\text{Amount (mg) of haloperidol } (C_{21}H_{23}ClFNO_2) = M_S \times A_T/A_S \times 2/5$$

M_S: Amount (mg) of haloperidol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 250 mL of this solution add 750 mL of methanol, and add 1.0 g of sodium lauryl sulfate to dissolve.

Flow rate: Adjust so that the retention time of haloperidol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of haloperidol are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloperidol is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Colored containers may be used.

Storage—Light-resistant.

Haloperidol Tablets

ハロペリドール錠

Haloperidol Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$: 375.86).

Method of preparation Prepare as directed under Tablets, with Haloperidol.

Identification To powdered Haloperidol Tablets, equivalent to 6 mg of Haloperidol, add 70 mL of 2-propanol, and heat on a water bath until to boiling while shaking. After cooling, add 2-propanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 2 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 219 nm and 223 nm and between 243 nm and 247 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Haloperidol Tablets add 5 mL of the mobile phase, disperse the particle with the aid of ultrasonic waves, add 30 mL of the mobile phase, and extract for 30 minutes with the aid of ultrasonic waves with occasional shaking. Shake for more 30 minutes, and add the mobile phase to make exactly 50 mL. Centrifuge the solution, pipet V mL of the supernatant liquid, equivalent to about 0.3 mg of haloperidol ($C_{21}H_{23}ClFNO_2$), add exactly 2 mL of the internal standard solution and the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in the mobile phase to make exactly 100 mL. Pipet 15 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution and the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of haloperidol to that of the internal standard.

$$\text{Amount (mg) of haloperidol } (C_{21}H_{23}ClFNO_2) = M_S \times Q_T/Q_S \times 1/V \times 3/4$$

M_S: Amount (mg) of haloperidol for assay taken

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 6700).

Operating conditions—

Proceed as detected in the operating condition in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately, and powder not less than 20 Haloperidol Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of haloperidol ($C_{21}H_{23}ClFNO_2$), add 10 mL of water, disperse the particle with the aid of ultrasonic waves, add exactly 20 mL of the internal standard solution, extract for 30 minutes with the aid of ultrasonic waves with occasional shaking, and add the mobile phase to make 100 mL. Centrifuge after shaking for more 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution and the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of haloperidol to that of the internal standard.

$$\text{Amount (mg) of haloperidol } (C_{21}H_{23}ClFNO_2) = M_S \times Q_T/Q_S \times 2/5$$

M_S: Amount (mg) of haloperidol for assay taken

Internal standard solution—A solution of diphenyl in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 250 mL of this solution add 750 mL of methanol and 1.0 g of sodium lauryl sulfate, and mix to dissolve.

Flow rate: Adjust so that the retention time of haloperidol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.

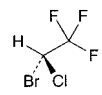
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant for the tablets without coating.

Halothane

ハロタン



and enantiomer

$C_2HBrClF_3$: 197.38

(2*RS*)-2-Bromo-2-chloro-1,1,1-trifluoroethane

[*151-67-7*]

Halothane contains not less than 0.008% and not more than 0.012% of Thymol as a stabilizer.

Description Halothane is a clear, colorless, and mobile liquid.

It is miscible with ethanol (95), with diethyl ether and with isooctane.

It is slightly soluble in water.

It is a volatile, nonflammable liquid, and setting fire to its

heated vapor does not support combustion.

It is affected by light.

Refractive index n_D^{20}: 1.369 – 1.371

Identification Transfer about 3 μL of Halothane to a gas cell having light path 10 cm in length, and determine the infrared absorption spectrum as directed in the gas sampling method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.872 – 1.877

Purity **(1)** Acidity or alkalinity—Shake 60 mL of Halothane with 60 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer, and use this as the sample solution. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color develops. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is produced.

(2) Halide and halogen—To 5 mL of the sample solution obtained in (1) add 1 drop of nitric acid and 0.20 mL of silver nitrate TS: no turbidity is produced. To 10 mL of the sample solution obtained in (1) add 1 mL of potassium iodide TS and 2 drops of starch TS, and allow to stand for 5 minutes: no blue color develops.

(3) Phosgene—Transfer 50 mL of Halothane to a dried 300-mL conical flask, suspend a strip of phosgene test paper vertically inside the flask with the lower end about 10 mm above the surface of the liquid, insert the stopper, and allow to stand at a dark place for 20 to 24 hours: the test paper shows no yellow color.

(4) Residue on evaporation—Pipet 50 mL of Halothane, evaporate on a water bath, and dry the residue at 105°C for 2 hours: the mass of the residue is not more than 1.0 mg.

(5) Volatile related substances—To 100 mL of Halothane add exactly 5.0 μL of the internal standard, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Gas Chromatography ⟨*2.02*⟩, and determine each peak area by the automatic integration method: the total area of the peaks other than halothane and the internal standard is not larger than the peak area of the internal standard.

Internal standard—1,1,2-Trichloro-1,2,2-trifluoroethane

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and 3 m in length, at the first 2 m from the injection port, having macrogol 400 coated in the ratio of 30% on siliceous earth for gas chromatography (180 to 250 μm in particle diameter), and at the remaining 1 m, having dinonyl phthalate coated in the ratio of 30% on siliceous earth for gas chromatography (180 to 250 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of the internal standard is 2 to 3 minutes.

Selection of column: Mix 3 mL of Halothane and 1 mL of the internal standard. Proceed with 1 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and halothane in this order with the resolution between these peaks being not less than 10.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of the internal standard obtained from 5 μL of the sample solution composes 30 to 70% of the full scale.

Time span of measurement: About 3 times as long as the retention time of halothane.

Distilling range ⟨*2.57*⟩ Not less than 95 vol% distils within a 1°C range between 49°C and 51°C.

Thymol To 0.50 mL of Halothane add 5.0 mL of isooctane and 5.0 mL of titanium (IV) oxide TS, shake vigorously for 30 seconds, and allow to stand: the separated upper layer has more color than the following control solution A, and has no more color than the following control solution B.

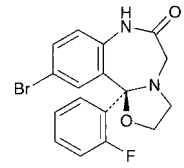
Control solution: Dissolve 0.225 g of thymol for assay in isooctane to make exactly 100 mL. To 10 mL each of this solution, accurately measured, add isooctane to make exactly 150 mL and 100 mL, respectively. Proceed with 0.50 mL each of these solutions in the same manner as Halothane, and use the separated upper layers so obtained as the control solution A and B, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 30°C.

Haloxazolam

ハロキサゾラム



and enantiomer

$C_{17}H_{14}BrFN_2O_2$: 377.21

(11b*RS*)-10-Bromo-11b-(2-fluorophenyl)-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one

[*59128-97-1*]

Haloxazolam, when dried, contains not less than 99.0% of haloxazolam ($C_{17}H_{14}BrFN_2O_2$).

Description Haloxazolam occurs as white, crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), sparingly soluble in acetonitrile, in methanol and in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 183°C (with decomposition).

Identification **(1)** Dissolve 10 mg of Haloxazolam in 10 mL of methanol, add 1 drop of hydrochloric acid: the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). To this solution add 1 mL of sodium hydroxide TS: the fluorescence disappears immediately.

(2) Prepare the test solution with 50 mg of Haloxazolam as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 20 mL of dilute sodium hydroxide TS and 1 mL of hydrogen peroxide (30) as an absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ for bromide and for fluoride.

(3) Determine the absorption spectrum of a solution of Haloxazolam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave-

lengths.

(4) Determine the infrared absorption spectrum of Haloxazolam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance ⟨2.24⟩ $E^{1\%}_{1\,cm}$ (247 nm): 390 – 410 (10 mg, methanol, 1000 mL).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Haloxazolam in 20 mL of ethanol (99.5): the solution is clear and colorless.

(2) Soluble halides—To 1.0 g of Haloxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as directed under Chloride Limit Test ⟨1.03⟩. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS.

(3) Heavy metals ⟨1.07⟩—Proceed with 1.0 g of Haloxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic ⟨1.11⟩—To 1.0 g of Haloxazolam in a decomposition flask add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat, repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution is colorless to pale yellow. After cooling, add 2 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution: the solution has no more color than the following control solution (not more than 2 ppm).

Control solution: Proceed in the same manner as above without using Haloxazolam, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and proceed in the same manner as the test solution.

(5) Related substances—Dissolve 0.10 g of Haloxazolam in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than haloxazolam from the sample solution is not larger than the peak area of the haloxazolam from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.2 g of boric acid and 7.5 g of potassium chloride in 900 mL of water, adjust the pH with triethylamine to 8.5, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust so that the retention time of haloxazolam is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of haloxazolam, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of haloxazolam obtained from 10 μL of this solution is equivalent to 8 to 12% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 10 mg each of Haloxazolam and cloxazolam in 200 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, haloxazolam and cloxazolam are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloxazolam is not more than 1.0%.

Loss on drying ⟨2.41⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g, platinum crucible).

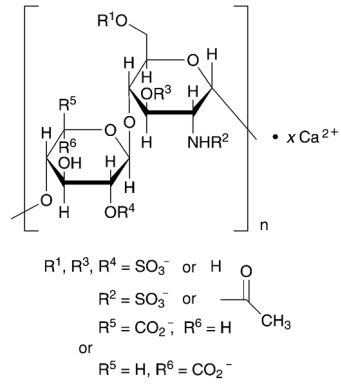
Assay Weigh accurately about 0.5 g of Haloxazolam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.72 mg of $C_{17}H_{14}BrFN_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Heparin Calcium

ヘパリンカルシウム



[37270-89-6]

Heparin Calcium is the calcium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid) obtained from the intestinal mucosa of healthy edible swine.

It prolongs the clotting time of blood.

It contains not less than 180 Heparin Units (anti-factor IIa activity) per mg, calculated on the dried basis, and not less than 8.0% and not more than 12.0% of calcium (Ca: 40.08).

Description Heparin Calcium occurs as a white to grayish

brown, powder or grains.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 10 mg of Heparin Calcium in 5 mL of water, and add 0.1 mL of 1 mol/L hydrochloric acid TS and 5 mL of toluidine blue O solution (1 in 20,000): a purple to red-purple color develops.

(2) Dissolve 1 mg each of Heparin Calcium and Heparin Sodium RS for Physicochemical Test in 1 mL of water, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention times for the major peaks from the sample solution and the standard solution are identical.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of mobile phase and flow rate: Proceed as directed under the operating conditions in Purity (9).

System suitability—

System performance: Dissolve 1.0 mg of Heparin Sodium RS for Physicochemical Test in 0.60 mL of water. Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water. Dissolve 1.0 mg of dermatan sulfate in 2.0 mL of water. To 90 μL of the solution of Heparin Sodium RS for Physicochemical Test add 30 μL each of the solutions of Over-sulfated Chondroitin Sulfate RS and dermatan sulfate, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, dermatan sulfate, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between the peaks of dermatan sulfate and heparin being not less than 1.0 and that between the peaks of heparin and over-sulfated chondroitin sulfate being not less than 1.5.

(3) A solution of 50 mg of Heparin Calcium in 5 mL of water responds to the Qualitative Tests <*1.09*> for calcium salt.

pH <*2.54*> Dissolve 1.0 g of Heparin Calcium in 100 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Heparin Calcium in 20 mL of water: the solution is clear. Determine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.05.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of Heparin Calcium. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Heavy metals <*1.07*>—Proceed with 0.5 g of Heparin Calcium according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(4) Barium—Dissolve 30 mg of Heparin Calcium in 3.0 mL of water, and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(5) Total nitrogen—Weigh accurately about 0.1 g of Heparin Calcium, previously dried, and perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is not more than 3.0%.

(6) Protein—(i) Sodium carbonate solution: To 4 volumes of a mixture of sodium hydroxide solution (1 in 100) and anhydrous sodium carbonate solution (1 in 20) (1:1) add 1 volume of water.

(ii) Copper sulfate solution: To 4 volumes of a mixture of copper (II) sulfate pentahydrate solution (1 in 80) and sodium tartrate dihydrate solution (149 in 5000) (1:1) add 1 volume of water.

(iii) Alkaline copper solution for heparin: Mix 50 volumes of the sodium carbonate solution and 1 volume of the copper sulfate solution. Prepare before using.

(iv) Procedure: Use a solution of Heparin Calcium (1 in 200) as the sample solution. Use a solution of bovine serum albumin (1 in 40,000) as the standard solution. To exactly 1 mL each of the sample solution and standard solution add exactly 5 mL of the alkaline copper solution for heparin, mix, and allow them to stand at room temperature for 10 minutes. To each of these solutions add exactly 0.5 mL of diluted Folin's TS (1 in 2), shake, allow them to stand at room temperature for 30 minutes, and centrifuge at room temperature. Determine the absorbances at 750 nm of the supernatant liquids as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank: the absorbance of the solution obtained from the sample solution is not more than that of the solution obtained from the standard solution.

(7) Nucleic acid—Dissolve 40 mg of Heparin Calcium in 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (93 in 50,000), and determine the absorbance of this solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.15.

(8) Over-sulfated chondroitin sulfate—Dissolve 20 mg of Heparin Calcium in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> (^{1}H) in accordance with the following conditions, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits no signal corresponding to *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.18 ± 0.05 ppm, the signal disappears when determining the spectrum of the sample solutions as directed under ^{1}H with ^{13}C-decoupling.

Operating conditions—

Spectrometer: 1.1. FT-NMR, Not less than 400 MHz.

Temperature: 25°C.

Spinning: off.

Number of data points: 32,768.

Spectral range: Signal of DHO ±6.0 ppm.

Flip angle: 90°.

Delay time: 20 seconds.

Dummy scans: 4.

Number of scans: S/N of the signal of *N*-acetyl proton signal of heparin is not less than 1000.

Window function: Exponential function (Line broadening factor = 0.2 Hz).

System suitability—

System performance: Dissolve 20 mg of Heparin Calcium in 0.40 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 1.0 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). To the solution of heparin calcium add 0.20 mL of the solution of Over-sulfated Chondroitin

Sulfate RS. When determining the spectrum of this solution under the above operating conditions, it exhibits the signal of *N*-acetyl proton of heparin and the signal of *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.04 ± 0.02 ppm and δ 2.18 ± 0.05 ppm, respectively.

(9) Related substances—Dissolve 2.0 mg of Heparin Calcium in 0.1 mL of water, and perform the test with exactly 20 μL of this solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions: it exhibits no peaks after the heparin peak.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 202 nm).

Column: A stainless steel column 2.0 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

Mobile phase B: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate and 106.4 g of lithium perchlorate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 3	90	10
3 – 15	90 → 0	10 → 100

Flow rate: 0.2 mL per minute.

Time span of measurement: About 2 times as long as the retention time of heparin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 10 mg of Heparin Sodium RS for Physicochemical Test in 0.40 mL of water, and use this solution as the heparin sodium standard stock solution. Separately, dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water, and use this solution as the over-sulfated chondroitin sulfate standard solution. To 60 μL of the heparin sodium standard stock solution add 3 μL of the over-sulfated chondroitin sulfate standard solution and 12 μL of water, and mix. When the procedureis run with 20 μL of the mixture under the above operating conditions, it exhibits an over-sulfated chondroitin sulfate peak.

System performance: To 120 μL of the heparin sodium standard stock solution add 30 μL of the over-sulfated chondroitin sulfate standard solution, mix and use this solution as the solution for system suitability test. When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of over-sulfated chondroitin sulfate is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 8% (50 mg, in vacuum, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.0030 EU/heparin Unit.

Anti-factor Xa activity to anti-factor IIa activity ratio The ratio of the anti-factor Xa activity determined by the following method to the anti-factor IIa activity obtained in the Assay, calculated by dividing the former with the later, is 0.9 – 1.1.

Anti-factor Xa activity determination

(i) Substrate solution: Dissolve 25 mg of *N*-benzoyl-L-isoleucyl-L-glutamyl(γ-OR)-glycyl-L-arginyl-*p*-nitroanilide hydrochloride in 33.3 mL of water.

(ii) Anti-thrombin solution: Dissolve human anti-thrombin in water so that each mL contains 1 IU. To 150 μL of this solution add 2250 μL of buffer solution.

(iii) Factor Xa solution: To 1200 μL of factor Xa TS add 1200 μL of buffer solution.

(iv) Buffer solution: Proceed as directed in the Assay (1).

(v) Stopping solution: Proceed as directed in the Assay (1).

(vi) Heparin standard solutions: Proceed as directed in the Assay (1). However, the standard solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(vii) Heparin sample solutions: Proceed as directed in the Assay (1). However, the sample solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(viii) Procedure: Transfer separately two 50-μL portions of each different dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer solution as the blank to 1.5-mL tubes. Warm these 21 tubes, anti-thrombin solution, factor Xa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2, S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 50 μL of anti-thrombin solution, mix, and warm at 37°C for exactly 4 minutes, add 100 μL of factor Xa solution, mix, and incubate for exactly 12 minutes. Then, add 100 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix immediately. Separately, to 50 μL of stopping solution add 100 μL of substrate solution, 100 μL of factor Xa solution, 50 μL of anti-thrombin solution and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{X_S} + B_{X_t}$, is obtained using y as log of the absorbance values, $_{X_S}$ as the concentration of the heparin standard solutions and $_{X_t}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept

A: Slope of regression expression of the heparin standard solution

B: Slope of regression expression of the heparin sample solution

Calculate anti-factor Xa activity per mg of Heparin Calcium by the following formula.

$$\text{Anti-factor Xa activity per mg of Heparin Calcium} = 100 \times R \times V/M$$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 anti-factor

Xa activity Units per mL
M: Amount (mg) of Heparin Calcium taken for the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{Xs} + B'_{Xt} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in the range of between -0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are performed as directed in the Assay (1). When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Assay (1) Heparin

(i) Substrate solution: Dissolve 25 mg of *H*-D-phenylalanyl-L-pipecolyl-L-arginyl-*p*-nitroanilide dihydrochloride in 32.0 mL of water.

(ii) Anti-thrombin solution (for heparin assay): Dissolve human anti-thrombin in water so that each mL contains 1 IU. Dilute this solution to an appropriate dilution factor of approximately more than 16 times with the buffer solution, and designate this solution as the anti-thrombin solution (for heparin assay). The dilution factor with the buffer solution is adjusted so that the absorbance of reaction solution with the blank solution (average of five tubes) is not more than 2.0, and that of reaction solution with S_4 (0.020 Unit/mL heparin standard solution) (average of two tubes) is not less than 0.2 and not more than 1.0 when the test is performed according to the Assay. The absorbance is measured with 1 cm light path in length.

(iii) Factor IIa solution: Add an equivalent volume of water to the buffer solution, and use this solution as the factor IIa diluent. Dissolve factor IIa in the factor IIa diluent to make a solution so that each mL contains 20 IU. Dilute this solution to an appropriate dilution factor of approximately less than 4 times with the factor IIa diluent, and designate this solution as the factor IIa solution. Adjust the dilution factor with the factor IIa diluent so that the absorbance of reaction solution with the blank solution (average of five tubes) is not more than 2.0, and that of reaction solution with S_4 (0.020 Unit/mL heparin standard solution) (average of two tubes) is not less than 0.2 and not more than 1.0 when the test is performed according to the Assay. The absorbance is measured with 1 cm light path in length.

(iv) Buffer solution: Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 10.2 g of sodium chloride, 2.8 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.0 g of polyethylene glycol 6000 in 800 mL of water, adjust to pH 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(v) Stopping solution: To 2 mL of acetic acid (100) add water to make 10 mL.

(vi) Heparin standard solutions: Dissolve Heparin Sodium RS in water so that each mL contains 100 Heparin Units, and use this solution as the standard stock solution. Dilute the standard stock solution with buffer solution so that each mL contains exactly 0.1 Heparin Units, and use this solution as the standard solution. Make heparin standard solutions S_1, S_2, S_3 and S_4 respectively by adding the standard solution to buffer solution as directed in the following table.

Heparin standard solution		Buffer solution (μL)	Standard solution (μL)
No.	Heparin concentration (Unit/mL)		
S_1	0.005	950	50
S_2	0.010	900	100
S_3	0.015	850	150
S_4	0.020	800	200

(vii) Heparin sample solutions: Weigh accurately an appropriate amount of Heparin Calcium, dissolve in water so that each mL contains about 100 Heparin Units, and use this solution as the sample stock solution. Dilute exactly the sample stock solution with buffer solution so that each mL contains 0.1 Heparin Units, and use this solution as the sample solution. Make heparin sample solutions T_1, T_2, T_3 and T_4 respectively by adding the sample solution to buffer solution as directed in the following table.

Heparin sample solution		Buffer solution (μL)	Sample solution (μL)
No.	Heparin concentration (Unit/mL)		
T_1	0.005	950	50
T_2	0.010	900	100
T_3	0.015	850	150
T_4	0.020	800	200

(viii) Procedure: Transfer separately two 50-μL portions of each dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer solution as the blank to 1.5-mL tubes. Warm these 21 tubes, anti-thrombin solution (for heparin assay), factor IIa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2, S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 100 μL of anti-thrombin solution (for heparin assay), mix, and warm at 37°C for exactly 4 minutes, add 25 μL of factor IIa solution, mix, and incubate for exactly 4 minutes. Then, add 50 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix. Separately, to 50 μL of stopping solution add 50 μL of substrate solution, 25 μL of factor IIa solution, 100 μL of anti-thrombin solution (for heparin assay) and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{Xs} + B_{Xt}$, is obtained using y as log of the absorbance values, $_{Xs}$ as the concentration of the heparin standard solutions and $_{Xt}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample

solution

Calculate Heparin Unit (anti-factor IIa activity) per mg of Heparin Calcium by the following formula.

Heparin Unit (anti-factor IIa activity) per mg of Heparin Calcium
$= 100 \times R \times V/M$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 Heparin Units (anti-factor IIa activity) per mL
M: Amount (mg) of Heparin Calcium taken for the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{Xs} + B'_{Xt} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in the range of between -0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are the following 3 items, (1), (2) and (3).

(1) Judgment on consistence of the intercept assumed from the two lines

When the regression expression, $y = I_s + A''_{Xs} + B''_{Xt} + I_{t\text{-}s}$, is obtained from the data of the heparin standard solutions and the heparin sample solutions except of the blank solution, a 90% confidence interval of the constant term, $I_{t\text{-}s}$, is between -0.2 and 0.2.

I_s: Intercept of the regression expression of the heparin standard solution
$I_{t\text{-}s}$: Difference of the intercepts assumed from the two lines

(2) Judgment on linearity

When the regression expression, $y = I_c + A'''_{Xs} + B'''_{Xt} + Q_{sXs}{}^2 + Q_{tXt}{}^2$, is obtained from the data of the heparin standard solutions and the heparin sample solutions, a 90% confidence interval of the secondary coefficients, Q_s and Q_t, is between -1000 and 1000.

Q_s: Secondary coefficient of the regression expression of the heparin standard solution
Q_t: Secondary coefficient of the regression expression of the heparin sample solution

(3) Judgment by checking if the relative potency obtained is within the range previously validated on this test method

The potency ratio obtained is not less than 0.8 and not more than 1.2.

When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

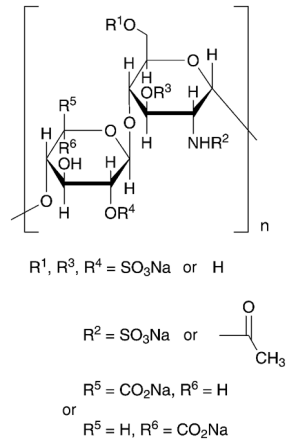
(2) Calcium: Weigh accurately about 50 mg of Heparin Calcium, dissolve in 20 mL of water, add 2 mL of 8 mol/L potassium hydroxide TS, allow to stand for 3 to 5 minutes with occasional shaking, add 0.1 g of NN indicator, and immediately titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.4008 mg of Ca

Containers and storage Containers—Tight containers.

Heparin Sodium

ヘパリンナトリウム



R^1, R^3, R^4 = SO_3Na or H

R^2 = SO_3Na or

R^5 = CO_2Na, R^6 = H
or
R^5 = H, R^6 = CO_2Na

[*9041-08-1*]

Heparin Sodium is a sodium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid) obtained from the intestinal mucosa of healthy edible swine.

It prolongs the clotting time of blood.

It contains not less than 180 Heparin Units (anti-factor IIa activity) per mg, calculated on the dried basis.

Description Heparin Sodium occurs as a white to grayish brown, powder or grains. It is odorless.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification Dissolve 1 mg each of Heparin Sodium and Heparin Sodium RS for Physicochemical Test in 1 mL of water, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the retention times for the major peaks from the sample solution and the standard solution are identical.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of mobile phase and flow rate: Proceed as directed under the operating conditions in Purity (7).

System suitability—

System performance: Dissolve 1.0 mg of Heparin Sodium RS for Physicochemical Test in 0.60 mL of water. Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water. Dissolve 1.0 mg of dermatan sulfate in 2.0 mL of water. To 90 μL of the solution of Heparin Sodium RS for Physicochemical Test add 30 μL each of the solutions of Over-sulfated Chondroitin Sulfate RS and dermatan sulfate, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, dermatan sulfate, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between the peaks of dermatan sulfate and heparin being not less than 1.0 and that between the peaks of heparin and over-sulfated chondroitin sulfate being not less than 1.5.

pH ⟨*2.54*⟩ The pH of a solution of 1.0 g of Heparin Sodium in 100 mL of water is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Heparin Sodium in 20 mL of water: the solution is clear and colorless to light yellow.

(2) Barium—Dissolve 30 mg of Heparin Sodium in 3.0 mL of water, and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(3) Total nitrogen—Weigh accurately about 0.1 g of Heparin Sodium, previously dried at 60°C for 3 hours under reduced pressure, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩: the amount of nitrogen (N: 14.01) is not more than 3.0%.

(4) Protein—(i) Sodium carbonate solution: To 4 volumes of a mixture of sodium hydroxide solution (1 in 100) and anhydrous sodium carbonate solution (1 in 20) (1:1) add 1 volume of water.

(ii) Copper sulfate solution: To 4 volumes of a mixture of copper (II) sulfate pentahydrate solution (1 in 80) and sodium tartrate dihydrate solution (149 in 5000) (1:1) add 1 volume of water.

(iii) Alkaline copper solution for heparin: Mix 50 volumes of the sodium carbonate solution and 1 volume of the copper sulfate solution. Prepare before using.

(iv) Procedure: Use a solution of Heparin Sodium (1 in 200) as the sample solution. Use a solution of bovine serum albumin (1 in 40,000) as the standard solution. To exactly 1 mL each of the sample solution and standard solution add exactly 5 mL of the alkaline copper solution for heparin, mix, and allow them to stand at room temperature for 10 minutes. To each of these solutions add exactly 0.5 mL of diluted Folin's TS (1 in 2), shake, and allow them to stand at room temperature for 30 minutes. Determine the absorbances at 750 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using water as the blank: the absorbance of the solution obtained from the sample solution is not more than that of the solution obtained from the standard solution.

(5) Nucleic acid—Dissolve 40 mg of Heparin Sodium in 10 mL of water, and determine the absorbance of this solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance is not more than 0.15.

(6) Over-sulfated chondroitin sulfate—Dissolve 20 mg of Heparin Sodium in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩ (^{1}H) in accordance with the following conditions, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits no signal corresponding to *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.15 ± 0.02 ppm, or the signal disappears when determining the spectrum of the sample solutions as directed under ^{1}H with ^{13}C-decoupling.

Operating conditions—

Spectrometer: 1.1. FT-NMR, Not less than 400 MHz.

Temperature: 25°C.

Spinning: off.

Number of data points: 32,768.

Spectral range: Signal of DHO ±6.0 ppm.

Flip angle: 90°.

Delay time: 20 seconds.

Dummy scans: 4.

Number of scans: S/N of the signal of *N*-acetyl proton signal of heparin is not less 1000.

Window function: Exponential function (Line broadening factor = 0.2 Hz).

System suitability—

System performance: Dissolve 20 mg of Heparin Sodium RS for Physicochemical Tests in 0.40 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 1.0 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). To the solution of Heparin Sodium RS for Physicochemical Tests add 0.20 mL of the solution of Over-sulfated Chondroitin Sulfate RS. When determining the spectrum of this solution under the above operating conditions, it exhibits the signal of *N*-acetyl proton of heparin and the signal of *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.04 ± 0.02 ppm and δ 2.15 ± 0.02 ppm, respectively.

(7) Related substances—Dissolve 2.0 mg of Heparin Sodium in 0.1 mL of water and perform the test with exactly 20 μL of this solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions: it exhibits no peaks after the heparin peak.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 202 nm).

Column: A stainless steel column 2.0 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

Mobile phase B: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate and 106.4 g of lithium perchlorate in 1000 mL of water, and adjust to a pH of 3.0 with diluted phosphoric acid (1 in 10).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 3	90	10
3 – 15	90 → 0	10 → 100

Flow rate: 0.2 mL per minute.

Time span of measurement: About 2 times as long as the retention time of heparin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 10 mg of Heparin Sodium RS for Physicochemical Test in 0.40 mL of water, and use this solution as the heparin sodium standard stock solution. Separately, dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water, and use this solution as the over-sulfated chondroitin sulfate standard solution. To 60 μL of the heparin sodium standard stock solution add 3 μL of the over-sulfated chondroitin sulfate standard solution and 12 μL of water, and mix. When the procedure is run with 20 μL of the mixture under the above

operating conditions, it exhibits a peak for over-sulfated chondroitin sulfate.

System performance: To 120 μL of the heparin sodium standard stock solution add 30 μL of the over-sulfated chondroitin sulfate standard solution, mix and use this solution as the solution for system suitability test. When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of over-sulfated chondroitin sulfate is not more than 2.0%.

(8) Galactosamine—Dissolve 2.4 mg of Heparin Sodium in 1.0 mL of a mixture of water and hydrochloric acid (7:5), and use this solution as the heparin sodium stock solution. Dissolve 8.0 mg of D-glucosamine hydrochloride in a mixture of water and hydrochloric acid (7:5) to make exactly 10 mL. Dissolve 8.0 mg of D-galactosamine hydrochloride in a mixture of water and hydrochloric acid (7:5) to make exactly 10 mL. To 99 volumes of the solution of D-glucosamine add 1 volume of the solution of D-galactosamine, and use this solution as the standard stock solution. Transfer 500 μL each of the heparin sodium stock solution and the standard stock solution to a glass-stoppered test tube, stopper tightly, and heat at 100°C for 6 hours. After cooling to room temperature, evaporate 100 μL each of the reaction solutions to dryness. Add 50 μL of methanol to each of the residues and evaporate to dryness at room temperature. Dissolve each of the residues in 10 μL of water, add 40 μL of aminobenzoate derivatization TS, and heat at 80°C for 1 hour. After cooling to room temperature, evaporate the reaction solutions to dryness. Add 200 μL of each of water and ethyl acetate to each of the residues, shake vigorously, and then centrifuge. After remove the upper layers, add 200 μL of ethyl acetate to each of the lower layers, shake vigorously, and then centrifuge. These lower layers are used as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the peak area ratio of galactosamine to glucosamine of the sample solution is not larger than that of the standard solution.

Operating conditions—

Detector: A fluorescence photometer (excitation wavelength: 305 nm; emission wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: To 100 mL of a mixture of water and trifluoroacetic acid (1000:1) add 100 mL of acetonitrile. Add 140 mL of the solution to 860 mL of a mixture of water and trifluoroacetic acid (1000:1).

Flow rate: 1.0 mL per minute.

Time span of measurement: About 50 minutes after injected.

System suitability—

Test for required detectability: Dissolve 8.0 mg of D-mannosamine hydrochloride in 10 mL of a mixture of water and hydrochloric acid (7:5), and use this solution as the mannosamine standard solution. Transfer 500 μL of a mixture of the standard stock solution and the mannosamine standard solution (100:1) to a glass-stoppered test tube, stopper tightly, and heat at 100°C for 6 hours. After cooling this solution to room temperature, evaporate 100 μL of the reaction solution to dryness. Add 50 μL of methanol to the residue and evaporate to dryness at room temperature. Dissolve the residue in 10 μL of water, add 40 μL of aminobenzoate derivatization TS, and heat at 80°C for 1 hour. After cooling to room temperature, evaporate the reaction solution to dryness. Add 200 μL each of water and ethyl acetate to the residue, shake vigorously, and then centrifuge. After removing the upper layer, add 200 μL of ethyl acetate to the lower layer, shake vigorously, and then centrifuge. The lower layer is used as the solution for system suitability test. When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the ratio of the peak area of galactosamine to that of glucosamine is 0.7 – 2.0%.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, glucosamine, mannosamine and galactosamine are eluted in this order with the resolutions between the peaks of glucosamine and mannosamine and between the peaks of mannosamine and galactosamine being not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratio of the peak area of galactosamine to that of glucosamine is not more than 4.0%.

Loss on drying <*2.41*> Not more than 10% (20 mg, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 40% (after drying, 20 mg).

Bacterial endotoxins <*4.01*> Less than 0.0030 EU/Heparin Unit.

Anti-factor Xa activity to anti-factor IIa activity ratio The ratio of the anti-factor Xa activity determined by the following method to the anti-factor IIa activity obtained in the Assay, calculated by dividing the former with the later, is 0.9 – 1.1.

Anti-factor Xa activity determination

(i) Substrate solution: Dissolve 25 mg of *N*-benzoyl-L-isoleucyl-L-glutamyl(γ-OR)-glycyl-L-arginyl-*p*-nitroanilide hydrochloride in 33.3 mL of water.

(ii) Anti-thrombin solution: Dissolve human anti-thrombin in water so that each mL contains 1 IU. To 150 μL of this solution add 2250 μL of buffer solution

(iii) Factor Xa solution: To 1200 μL of factor Xa TS add 1200 μL of buffer solution.

(iv) Buffer solution: Proceed as directed in the Assay.

(v) Stopping solution: Proceed as directed in the Assay.

(vi) Heparin standard solutions: Proceed as directed in the Assay. However, the standard solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(vii) Heparin sample solutions: Proceed as directed in the Assay. However, the sample solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(viii) Procedure: Transfer separately two 50-μL portions of each dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer solution as the blank to 1.5 mL-tubes. Warm these 21 tubes, anti-thrombin solution, factor Xa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2,

S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 50 μL of anti-thrombin solution, mix, and warm at 37°C for exactly 4 minutes, add 100 μL of factor Xa solution, mix, and incubate for exactly 12 minutes. Then, add 100 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix immediately. Separately, to 50 μL of stopping solution add 100 μL of substrate solution, 100 μL of factor Xa solution, 50 μL of anti-thrombin solution and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{Xs} + B_{Xt}$, is obtained using y as log of the absorbance values, $_{Xs}$ as the concentration of the heparin standard solutions and $_{Xt}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample solution

Calculate anti-factor Xa activity per mg of Heparin Sodium by the following formula.

$$\text{Anti-factor Xa activity per mg of Heparin Sodium} = 100 \times R \times V/M$$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 anti-factor Xa activity Units per mL
M: Amount (mg) of Heparin Sodium taken for the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{Xs} + B'_{Xt} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in a range of between -0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are performed as directed in the Assay. When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Assay

(i) Substrate solution: Dissolve 25 mg of *H*-D-phenylalanyl-L-pipecolyl-L-arginyl-*p*-nitroanilide dihydrochloride in 32.0 mL of water.

(ii) Anti-thrombin solution (for heparin assay): Dissolve human anti-thrombin in water so that each mL contains 1 IU. Dilute this solution to an appropriate dilution factor of approximately more than 16 times with the buffer solution, and designate this solution as the anti-thrombin solution (for heparin assay). The dilution factor with the buffer solution is adjusted so that the absorbance of reaction solution with the blank solution (average of five tubes) is not more than 2.0, and that of reaction solution with S_4 (0.020 Unit/mL heparin standard solution) (average of two tubes) is not less than 0.2 and not more than 1.0 when the test is performed according to the Assay. The absorbance is measured with 1 cm light path in length.

(iii) Factor IIa solution: Add an equivalent volume of water to the buffer solution, and use this solution as the factor IIa diluent. Dissolve factor IIa in the factor IIa diluent to make a solution so that each mL contains 20 IU. Dilute this solution to an appropriate dilution factor of approximately less than 4 times with the factor IIa diluent, and designate this solution as the factor IIa solution. Adjust the dilution factor with the factor IIa diluent so that the absorbance of reaction solution with the blank solution (average of five tubes) is not more than 2.0, and that of reaction solution with S_4 (0.020 Unit/mL heparin standard solution) (average of two tubes) is not less than 0.2 and not more than 1.0 when the test is performed according to the Assay. The absorbance is measured with 1 cm light path in length.

(iv) Buffer solution: Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 10.2 g of sodium chloride, 2.8 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.0 g of polyethylene glycol 6000 in 800 mL of water, adjust to pH 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(v) Stopping solution: To 2 mL of acetic acid (100) add water to make 10 mL.

(vi) Heparin standard solutions: Dissolve Heparin Sodium RS in water so that each mL contains 100 Heparin Units, and use this solution as the standard stock solution. Dilute the standard stock solution with buffer solution so that each mL contains exactly 0.1 Heparin Units, and use this solution as the standard solution. Make heparin standard solutions S_1, S_2, S_3 and S_4 respectively by adding the standard solution to buffer solution as directed in the following table.

Heparin standard solution		Buffer solution (μL)	Standard solution (μL)
No.	Heparin concentration (Unit/mL)		
S_1	0.005	950	50
S_2	0.010	900	100
S_3	0.015	850	150
S_4	0.020	800	200

(vii) Heparin sample solutions: Weigh accurately an appropriate amount of Heparin Sodium, dissolve in water so that each mL contains about 100 Heparin Units, and use this solution as the sample stock solution. Dilute exactly the sample stock solution with buffer solution so that each mL contains 0.1 Heparin Units, and use this solution as the sample solution. Make heparin sample solutions T_1, T_2, T_3 and T_4 respectively by adding the sample solution to buffer solution as directed in the following table.

Heparin sample solution		Buffer solution (μL)	Sample solution (μL)
No.	Heparin concentration (Unit/mL)		
T_1	0.005	950	50
T_2	0.010	900	100
T_3	0.015	850	150
T_4	0.020	800	200

(viii) Procedure: Transfer separately two 50-μL portions of each dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer

solution as the blank to 1.5 mL-tubes. Warm these 21 tubes, anti-thrombin solution (for heparin assay), factor IIa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2, S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 100 μL of anti-thrombin solution (for heparin assay), mix, and warm at 37°C for exactly 4 minutes, add 25 μL of factor IIa solution, mix, and incubate for exactly 4 minutes. Then, add 50 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix. Separately, to 50 μL of stopping solution add 50 μL of substrate solution, 25 μL of factor IIa solution, 100 μL of anti-thrombin solution (for heparin assay) and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{X_S} + B_{X_t}$, is obtained using y as log of the absorbance values, $_{X_S}$ as the concentration of the heparin standard solutions and $_{X_t}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample solution

Calculate Heparin Unit (anti-factor IIa activity) per mg of Heparin Sodium by the following formula.

Heparin Unit (anti-factor IIa activity) per mg of Heparin Sodium
$= 100 \times R \times V/M$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 Heparin Units (anti-factor IIa activity) per mL
M: Amount (mg) of Heparin Sodium taken for the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{X_S} + B'_{X_t} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in the range of between −0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are the following 3 items, (1), (2) and (3).

(1) Judgment on consistence of the intercept assumed from the two lines

When the regression expression, $y = I_s + A''_{X_S} + B''_{X_t} + I_{t\text{-}s}$, is obtained from the data of the heparin standard solution and the heparin sample solution except of the blank solution, a 90% confidence interval of the constant term, $I_{t\text{-}s}$, is between −0.2 and 0.2.

I_s: Intercept of the regression expression of the heparin standard solution
$I_{t\text{-}s}$: Difference of the intercepts assumed from the two lines

(2) Judgment on linearity

When the regression expression, $y = I_c + A'''_{X_S} + B'''_{X_t} + Q_{sX_S}{}^2 + Q_{tX_t}{}^2$, is obtained from the data of the heparin standard solution and the heparin sample solution, a 90% confidence interval of the secondary coefficients, Q_s and Q_t, is between −1000 and 1000.

Q_s: Secondary coefficient of the regression expression of the heparin standard solution
Q_t: Secondary coefficient of the regression expression of the heparin sample solution

(3) Judgment by checking if the relative potency obtained is within the range previously validated on this test method

The potency ratio obtained is not less than 0.8 and not more than 1.2.

When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Containers and storage Containers—Tight containers.

Heparin Sodium Injection

ヘパリンナトリウム注射液

Heparin Sodium Injection is an aqueous injection.

It contains not less than 90% and not more than 110% of the labeled heparin Units.

Method of preparation Dissolve Heparin Sodium in Isotonic Sodium Chloride Solution and prepare as directed under Injections.

Description Heparin Sodium Injection is a clear, colorless to light yellow liquid.

pH ⟨*2.54*⟩ 5.5 – 8.0

Purity Barium—Measure exactly a volume of Heparin Sodium Injection, equivalent to 3000 Units of Heparin Sodium, add water to make 3.0 mL and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.0030 EU/unit.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Proceed as directed in the Assay under Heparin Sodium, replacing (vii) Heparin sample solutions and (ix) Calculations with the following.

(vii) Heparin sample solutions: Take exactly an appropriate amount of Heparin Sodium Injection, dilute exactly with buffer solution so that each mL contains 0.1 Heparin Units, and use this solution as the sample solution. Make heparin sample solutions T_1, T_2, T_3 and T_4 respectively by adding the sample solution to buffer solution as directed in the following table.

Heparin sample solution		Buffer solution (μL)	Sample solution (μL)
No.	Heparin concentration (Unit/mL)		
T_1	0.005	950	50
T_2	0.010	900	100
T_3	0.015	850	150
T_4	0.020	800	200

(ix) Calculations: When the regression expression, $y = I_c + A_{Xs} + B_{Xt}$, is obtained using y as log of the absorbance values, $_{Xs}$ as the concentration of the heparin standard solutions and $_{Xt}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample solution

Calculate Heparin Units (anti-factor IIa activity) in 1 mL of Heparin Sodium Injection by the following formula.

Heparin Units (anti-factor IIa activity) in 1 mL of Heparin Sodium Injection
$= 0.1 \times R \times V/a$

V: Total volume (mL) of the sample solution prepared as containing 0.1 Heparin Units (anti-factor IIa activity) per mL
a: Amount (mL) of Heparin Sodium Injection taken for the sample solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{Xs} + B'_{Xt} + D$, where D is a constant term showing the difference between the intercepts assumed from the measurement of the blank and the two lines, is not in the range of between -0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are followed as directed in the Assay under Heparin Sodium. When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Adsorbed Hepatitis B Vaccine

沈降 B 型肝炎ワクチン

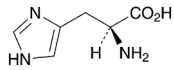
Adsorbed Hepatitis B Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing a surface antigen of hepatitis B virus to make the HBs antigen insoluble.

It conforms to the requirements of Adsorbed Hepatitis B Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Hepatitis B Vaccine becomes a homogeneous, whitish turbid liquid on shaking.

L-Histidine

L-ヒスチジン



$C_6H_9N_3O_2$: 155.15
(2*S*)-2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid
[*71-00-1*]

L-Histidine contains not less than 99.0% and not more than 101.0% of L-histidine ($C_6H_9N_3O_2$), calculated on the dried basis.

Description L-Histidine occurs as white, crystals or crystalline powder, having a slight bitter taste.

It is freely soluble in formic acid, and soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

It shows crystal polymorphism.

Identification Determine the infrared absorption spectrum of L-Histidine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample with a little amount of water, evaporate the water at 60°C under reduced pressure, dry the residue, and perform the test.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: $+11.8 - +12.8°$ (5.5 g calculated on the dried basis, 6 mol/L hydrochloric acid TS, 50 mL, 100 mm).

pH <*2.54*> The pH of a solution of 1.0 g of L-Histidine in 50 mL of water is between 7.0 and 8.5.

Purity **(1)** Clarity and color of solution—A solution of 0.40 g of L-Histidine in 20 mL of water is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Histidine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Histidine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Histidine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Dissolve 1.0 g of L-Histidine in 30 mL of water by warming. To this solution add 2.4 mL of dilute hydrochloric acid, 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Histidine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Histidine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for

thin-layer chromatography, develop the plate with a mixture of 1-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) to the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot obtained from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

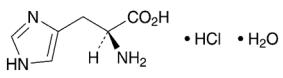
Assay Weigh accurately about 0.15 g of L-Histidine, dissolve in 2 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.52 mg of $C_6H_9N_3O_2$

Containers and storage Containers—Tight containers.

L-Histidine Hydrochloride Hydrate

L-ヒスチジン塩酸塩水和物



$C_6H_9N_3O_2.HCl.H_2O$: 209.63
(2*S*)-2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid monohydrochloride monohydrate
[*5934-29-2*]

L-Histidine Hydrochloride Hydrate contains not less than 99.0% and not more than 101.0% of L-histidine hydrochloride ($C_6H_9N_3O_2.HCl$: 191.62), calculated on the anhydrous basis.

Description L-Histidine Hydrochloride Hydrate occurs as white crystals or a white crystalline powder. It has an acid taste at first, and a slight bitter taste later.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

Identification **(1)** Determine the infrared absorption spectrum of L-Histidine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Histidine Hydrochloride Hydrate (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +9.2 – +10.6° (5.5 g calculated on the anhydrous basis, 6 mol/L hydrochloric acid TS, 50 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution of 1.0 g of L-Histidine Hydrochloride Hydrate in 10 mL of water is between 3.5 and 4.5.

Purity **(1)** Clarity and color of solution—A solution of 1.0 g of L-Histidine Hydrochloride Hydrate in 10 mL of water is clear and colorless.

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.6 g of L-Histidine Hydrochloride Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(3) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Histidine Hydrochloride Hydrate. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of L-Histidine Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Iron ⟨*1.10*⟩—Prepare the test solution with 1.0 g of L-Histidine Hydrochloride Hydrate according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.10 g of L-Histidine Hydrochloride Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) to the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot obtained from the standard solution.

Water ⟨*2.48*⟩ 7.2 – 10.0% (0.12 g, volumetric titration, direct titration, using a mixture of methanol for water determination and formamide for water determination (2:1) instead of methanol for water determination).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

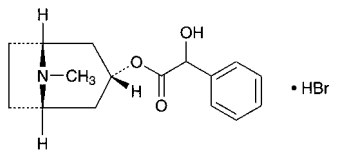
Assay Weigh accurately about 0.1 g of L-Histidine Hydrochloride Hydrate, dissolve in 3 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate ⟨*2.50*⟩ the excess of perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS
= 9.581 mg of $C_6H_9N_3O_2.HCl$

Containers and storage Containers—Tight containers.

Homatropine Hydrobromide

ホマトロピン臭化水素酸塩



$C_{16}H_{21}NO_3$.HBr: 356.25
(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl [(2*RS*)-2-hydroxy-2-phenyl]acetate monohydrobromide
[*51-56-9*]

Homatropine Hydrobromide contains not less than 99.0% of homatropine hydrobromide ($C_{16}H_{21}NO_3$.HBr), calculated on the dried basis.

Description Homatropine Hydrobromide occurs as white, crystals or crystalline powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is affected by light.

Melting point: about 214°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Homatropine Hydrobromide (1 in 20) add 2 to 3 drops of iodine TS: a brown precipitate is produced.

(2) Dissolve 0.05 g of Homatropine Hydrobromide in 5 mL of water, and add 3 mL of 2,4,6-trinitrophenol TS: a yellow precipitate is produced. Filter the precipitate, wash with five 10-mL portions of water, and dry at 105°C for 2 hours: it melts <*2.60*> between 184°C and 187°C.

(3) A solution of Homatropine Hydrobromide (1 in 20) responds to the Qualitative Tests <*1.09*> for bromide.

Purity **(1)** Acidity—Dissolve 1.0 g of Homatropine Hydrobromide in 20 mL of water, and add 0.40 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(2) Atropine, hyoscyamine and scopolamine—To 10 mg of Homatropine Hydrobromide add 5 drops of nitric acid, evaporate on a water bath to dryness, and cool. Dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: no red-purple color is produced.

(3) Related substances—Dissolve 0.15 g of Homatropine Hydrobromide in 3 mL of water, and use this solution as the sample solution.

(i) To 1 mL of the sample solution add 2 to 3 drops of tannic acid TS: no precipitate is produced.

(ii) To 1 mL of the sample solution add 2 to 3 drops each of dilute hydrochloric acid and platinic chloride TS: no precipitate is produced.

Loss on drying <*2.41*> Not more than 1.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.2 g).

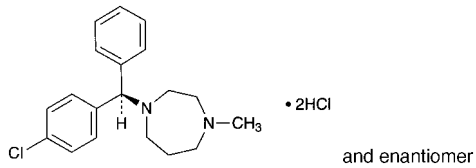
Assay Dissolve by warming about 0.4 g of Homatropine Hydrobromide in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 35.63 mg of $C_{16}H_{21}NO_3$.HBr

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Homochlorcyclizine Hydrochloride

ホモクロルシクリジン塩酸塩



$C_{19}H_{23}ClN_2$.2HCl: 387.77
1-[(*RS*)-(4-Chlorophenyl)(phenyl)methyl]-4-methylhexahydro-1*H*-1,4-diazepine dihydrochloride
[*1982-36-1*]

Homochlorcyclizine Hydrochloride, when dried, contains not less than 98.0% of homochlorcyclizine hydrochloride ($C_{19}H_{23}ClN_2$.2HCl).

Description Homochlorcyclizine Hydrochloride occurs as white to pale brown, crystals or powder.

It is very soluble in water, freely soluble in acetic acid (100), slightly soluble in ethanol (99.5), and very slightly soluble in acetonitrile and in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is hygroscopic.

It is colored slightly by light.

A solution of Homochlorcyclizine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 227°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Homochlorcyclizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Homochlorcyclizine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Homochlorcyclizine Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Homochlorcyclizine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Homochlorcyclizine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas by the automatic integration method: the areas of the peaks other than homochlorcyclizine obtained from the sample solution are not larger than 1/2 times the peak area of homochlorcyclizine obtained from the standard solution, and the total area of the peaks other than homochlorcyclizine from the sample solution is

not larger than the peak area of homochlorcyclizine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 223 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and perchloric acid (134:66:1).

Flow rate: Adjust so that the retention time of homochlorcyclizine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of homochlorcyclizine.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of homochlorcyclizine obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 5 mg each of Homochlorcyclizine Hydrochloride and methyl parahydroxybenzoic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoic acid and homochlorcyclizine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of homochlorcyclizine is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, 110°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Homochlorcyclizine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 19.39 mg of $C_{19}H_{23}ClN_2.2HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Human Normal Immunoglobulin

人免疫グロブリン

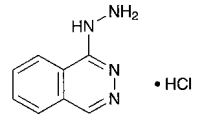
Human Normal Immunoglobulin is a liquid for injection containing immunoglobulin G in serum globulins of humans.

It conforms to the requirements of Human Normal Immunoglobulin in the Minimum Requirements for Biological Products.

Description Human Normal Immunoglobulin is a clear, colorless or yellow-brown liquid.

Hydralazine Hydrochloride

ヒドララジン塩酸塩



$C_8H_8N_4.HCl$: 196.64

Phthalazin-1-ylhydrazine monohydrochloride

[*304-20-1*]

Hydralazine Hydrochloride, when dried, contains not less than 98.0% of hydralazine hydrochloride ($C_8H_8N_4.HCl$).

Description Hydralazine Hydrochloride occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 275°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Hydralazine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Hydralazine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Hydralazine Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the pH of the solution is between 3.5 and 4.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the solution is clear, and colorless or pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Hydralazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 8 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Hydralazine Hydrochloride, previously dried, transfer it to a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, add 5 mL of chloroform, and titrate ⟨*2.50*⟩ with 0.05 mol/L potassium iodate VS while shaking until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color no more reappears in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4.HCl$

Containers and storage Containers—Tight containers.

Hydralazine Hydrochloride for Injection

注射用ヒドララジン塩酸塩

Hydralazine Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 99.0% and not more than 113.0% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4$.HCl: 196.64).

Method of preparation Prepare as directed under Injections, with Hydralazine Hydrochloride.

Description Hydralazine Hydrochloride for Injection occurs as a white to pale yellow powder or mass. It is odorless, and has a bitter taste.

Identification Determine the absorption spectrum of a solution of Hydralazine Hydrochloride for Injection (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm, and between 313 nm and 317 nm.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Hydralazine Hydrochloride for Injection in 50 mL of water: the pH of this solution is between 3.5 and 4.5.

Bacterial endotoxins ⟨*4.01*⟩ Less than 5.0 EU/mg.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test. (*T*: 106.0%)

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the contents of not less than 10 samples of Hydralazine Hydrochloride for Injection. Weigh accurately about 0.15 g of the contents, transfer it to a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4$.HCl

Containers and storage Containers—Hermetic containers.

Hydralazine Hydrochloride Powder

ヒドララジン塩酸塩散

Hydralazine Hydrochloride Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4$.HCl: 196.64).

Method of preparation Prepare as directed under Granules or Powders, with Hydralazine Hydrochloride.

Identification Weigh a portion of Hydralazine Hydrochloride Powder, equivalent to 25 mg of Hydralazine Hydrochloride, add 100 mL of water, shake well, and filter, if necessary. Add water to 2 mL of this solution to make 50 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm, and between 313 nm and 317 nm.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Hydralazine Hydrochloride Powder is not less than 85%.

Start the test with an accurately weighed amount of Hydralazine Hydrochloride Powder, equivalent to about 50 mg of hydralazine hydrochloride ($C_8H_8N_4$.HCl), withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 260 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of hydralazine hydrochloride ($C_8H_8N_4$.HCl)
$= M_S/M_T \times A_T/A_S \times 1/C \times 180$

M_S: Amount (mg) of hydralazine hydrochloride for assay taken
M_T: Amount (g) of the Hydralazine Hydrochloride Powder taken
C: Labeled amount (mg) of hydralazine hydrochloride ($C_8H_8N_4$.HCl) in 1 g

Assay Weigh accurately a portion of Hydralazine Hydrochloride Powder, equivalent to about 0.15 g of hydralazine hydrochloride ($C_8H_8N_4$.HCl), transfer it to a glass-stoppered flask, add 25 mL of water, shake well, add 25 mL of hydrochloric acid, cool to room temperature, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4$.HCl

Containers and storage Containers—Tight containers.

Hydralazine Hydrochloride Tablets

ヒドララジン塩酸塩錠

Hydralazine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4$.HCl: 196.64).

Method of preparation Prepare as directed under Tablets, with Hydralazine Hydrochloride.

Identification Weigh a quantity of powdered Hydralazine Hydrochloride Tablets, equivalent to 25 mg of Hydralazine Hydrochloride, add 100 mL of water, mix well, and filter if necessary. To 2 mL of this solution add water to make 50 mL, and determine the absorption spectrum of this solution

as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm and between 313 nm and 317 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Hydralazine Hydrochloride Tablets add 25 mL of 0.1 mol/L hydrochloric acid TS, disperse the tablet into a small particles using ultrasonic waves, then shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 10 μg of hydralazine hydrochloride ($C_8H_8N_4$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 260 nm, A_{T1} and A_{S1}, and at 350 nm, A_{T2} and A_{S2}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of hydralazine hydrochloride ($C_8H_8N_4$.HCl)
$= M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times V'/V \times 1/50$

M_S: Amount (mg) of hydralazine hydrochloride for assay taken

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Hydralazine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Hydralazine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of hydralazine hydrochloride ($C_8H_8N_4$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of hydralazine hydrochloride ($C_8H_8N_4$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of hydralazine hydrochloride for assay taken
C: Labeled amount (mg) of hydralazine hydrochloride ($C_8H_8N_4$.HCl) in 1 tablet

Assay Weigh accurately not less than 20 Hydralazine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of hydralazine hydrochloride ($C_8H_8N_4$.HCl), transfer it to a glass-stoppered flask, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4$.HCl

Containers and storage Containers—Tight containers.

Hydrochloric Acid

塩酸

Hydrochloric Acid contains not less than 35.0% and not more than 38.0% of hydrogen chloride (HCl: 36.46).

Description Hydrochloric Acid is a colorless liquid having a pungent odor.

It is fuming but ceases to fume when it is diluted with 2 volumes of water.

Specific gravity d_{20}^{20}: about 1.18.

Identification **(1)** Allow a glass stick wet with ammonia TS to come near the surface of Hydrochloric Acid: a remarkable white smoke evolves.

(2) A solution of Hydrochloric Acid (1 in 100) changes blue litmus paper to red, and responds to the Qualitative Tests <1.09> for chloride.

Purity **(1)** Sulfate <1.14>—To 15 mL of Hydrochloric Acid add water to make 50 mL, and use this solution as the sample solution. To 3.0 mL of the sample solution add 5 mL of water and 5 drops of barium chloride TS, and allow to stand for 1 hour: no turbidity is produced.

(2) Sulfite—To 3.0 mL of the sample solution obtained in (1) add 5 ml of water and 1 drop of iodine TS: the color of iodine TS does not disappear.

(3) Bromide or iodide—Place 10 mL of the sample solution obtained in (1) in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of 0.002 mol/L potassium permanganate VS, and shake well: the chloroform layer remains colorless.

(4) Bromine or chlorine—Place 10 mL of the sample solution obtained in (1) in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform, and shake for 1 minute: the chloroform layer remains free from a purple color.

(5) Heavy metals <1.07>—Evaporate 5 mL of Hydrochloric Acid on a water bath to dryness, and add 2 mL of dilute acetic acid and water to the residue to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follons: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.7 mL of Hydrochloric Acid according to Method 1, and perform the test (not more than 1 ppm).

(7) Mercury—Dilute 20 mL of Hydrochloric Acid with water to make exactly 100 mL, and use the solution as the sample solution. Perform the test with the sample solution as directed under Atomic Absorption Spectrophotometry <2.23> (cold vapor type). Place the sample solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the spectrophotometer, circulate air, and determine the absorbance A_T of the sample solution after the recorder reading has risen rapidly, and becomes constant at a wavelength of 253.7 nm. On the other hand, to 8 mL of Standard Mercury Solution add water to make exactly 100 mL, and determine the absorbance A_S of the solution ob-

tained by the same procedure as used for the sample solution: A_T is smaller than A_S (not more than 0.04 ppm).

Residue on ignition ⟨*2.44*⟩ Pipet 10 mL of Hydrochloric Acid, add 2 drops of sulfuric acid, evaporate to dryness, and ignite: not more than 1.0 mg of residue remains.

Assay Weigh accurately a glass-stoppered flask containing 20 mL of water, add about 3 mL of Hydrochloric Acid, and weigh accurately again. Dilute with 25 mL of water, and titrate ⟨*2.50*⟩ with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

Each mL of 1 mol/L sodium hydroxide VS
= 36.46 mg of HCl

Containers and storage Containers—Tight containers.

Dilute Hydrochloric Acid

希塩酸

Dilute Hydrochloric Acid contains not less than 9.5 w/v% and not more than 10.5 w/v% of hydrogen chloride (HCl: 36.46).

Description Dilute Hydrochloric Acid is a colorless liquid. It is odorless and has a strong acid taste.

Specific gravity d_{20}^{20}: about 1.05.

Identification A solution of Dilute Hydrochloric Acid (1 in 30) changes blue litmus paper to red and responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Purity **(1)** Sulfate—To 3.0 mL of Dilute Hydrochloric Acid add 5 mL of water and 5 drops of barium chloride TS, and allow to stand for 1 hour: no turbidity is produced.

(2) Sulfite—To 3.0 mL of Dilute Hydrochloric Acid add 5 mL of water and 1 drop of iodine TS: the color of iodine TS does not disappear.

(3) Bromide or iodide—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of 0.002 mol/L potassium permanganate VS, and shake well: the chloroform layer remains colorless.

(4) Bromine or chlorine—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform, and shake for 1 minute: the chloroform layer remains free from a purple color.

(5) Heavy metals ⟨*1.07*⟩—Evaporate 9.5 mL of Dilute Hydrochloric Acid on a water bath to dryness, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 3 ppm).

(6) Arsenic ⟨*1.11*⟩—Prepare the test solution with 4.0 mL of Dilute Hydrochloric Acid according to Method 1, and perform the test (not more than 0.5 ppm).

(7) Mercury—Dilute 80 mL of Dilute Hydrochloric Acid with water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ (cold vapor type). Place the sample solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the spectrophotometer, circulate air, and determine the absorbance A_T of the sample solution after the recorder reading has risen rapidly and become constant at a wavelength of 253.7 nm. On the other hand, to 8 mL of Standard Mercury Solution add water to make exactly 100 mL, and determine the absorbance A_S of the solution obtained by the same procedure as used for the sample solution: A_T is smaller than A_S (not more than 0.01 ppm).

Residue on ignition ⟨*2.44*⟩ Pipet 10 mL of Dilute Hydrochloric Acid, add 2 drops of sulfuric acid, evaporate to dryness, and ignite: the mass of the residue is not more than 1.0 mg.

Assay Measure exactly 10 mL of Dilute Hydrochloric Acid, and dilute with 20 mL of water. Titrate ⟨*2.50*⟩ with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

Each mL of 1 mol/L sodium hydroxide VS
= 36.46 mg of HCl

Containers and storage Containers—Tight containers.

Hydrochloric Acid Lemonade

塩酸リモナーデ

Method of preparation

Dilute Hydrochloric Acid	5 mL
Simple Syrup	80 mL
Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 mL

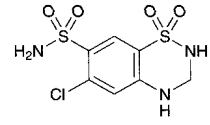
Prepare before use as directed under Lemonades, with the above ingredients.

Description Hydrochloric Acid Lemonade is a clear, colorless liquid. It has a sweet, cool, acid taste.

Containers and storage Containers—Tight containers.

Hydrochlorothiazide

ヒドロクロロチアジド



$C_7H_8ClN_3O_4S_2$: 297.74
6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide
[*58-93-5*]

Hydrochlorothiazide, when dried, contains not less than 99.0% of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Description Hydrochlorothiazide occurs as white, crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone, sparingly soluble in acetonitrile, very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: about 267°C (with decomposition).

Identification **(1)** To 5 mg of Hydrochlorothiazide add 5 mL of chromotropic acid TS, and allow to stand for 5

minutes: a purple color develops.

(2) Fuse a mixture of 0.1 g of Hydrochlorothiazide and 0.5 g of sodium carbonate decahydrate cautiously: the gas evolved changes moistened red litmus paper to blue. After cooling, crush with a glass rod, add 10 mL of water, stir, and filter. To 4 mL of the filtrate add 2 drops of hydrogen peroxide (30), 5 mL of diluted hydrochloric acid (1 in 5) and 2 to 3 drops of barium chloride TS: a white precipitate is produced.

(3) To 4 mL of the filtrate obtained in (2) add 5 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white precipitate is produced.

(4) Dissolve 12 mg of Hydrochlorothiazide in 100 mL of sodium hydroxide TS. Dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Hydrochlorothiazide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.036%).

(2) Sulfate <*1.14*>—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Hydrochlorothiazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Primary aromatic amines—Dissolve 80 mg of Hydrochlorothiazide in acetone to make exactly 100 mL. Measure exactly 1 mL of the solution, add 3.0 mL of dilute hydrochloric acid, 3.0 mL of water and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. Shake this solution with 1.0 mL of ammonium amidosulfate TS, allow to stand for 3 minutes, then add 1.0 mL of *N*,*N*-diethyl-*N*′-1-naphthylenediamine oxalate TS, shake, and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 1.0 mL of acetone in the same manner as the blank: the absorbance at 525 nm is not more than 0.10.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Hydrochlorothiazide and Hydrochlorothiazide RS, previously dried, and dissolve in 150 mL of the mobile phase, add exactly 10 mL each of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrochlorothiazide to that of the internal standard.

Amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Hydrochlorothiazide RS taken

Internal standard solution—A solution of 4-aminoacetophenone in acetonitrile (9 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.1 mol/L sodium dihydrogen phosphate TS (pH 3.0) and acetonitrile (9:1).

Flow rate: Adjust so that the retention time of hydrochlorothiazide is about 10 minutes.

System suitability—

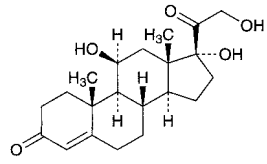
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrochlorothiazide and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrochlorothiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Hydrocortisone

ヒドロコルチゾン



$C_{21}H_{30}O_5$: 362.46

11β,17,21-Trihydroxypregn-4-ene-3,20-dione

[*50-23-7*]

Hydrocortisone, when dried, contains not less than 97.0% and not more than 102.0% of hydrocortisone ($C_{21}H_{30}O_5$).

Description Hydrocortisone occurs as a white crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in 1,4-dioxane, and very slightly soluble in water.

Melting point: 212 – 220°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone: the solution shows a yellow-green fluorescence immediately, and the color of the solution changes gradually from orange to dark red. Dilute carefully the solution with 10 mL of water: the color changes through yellow to orange-yellow with green fluorescence, and a small amount of a flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone in 1 mL of metha-

nol, add 1 mL of Fehling's TS, and heat: a red precipitate is formed.

(3) Determine the infrared absorption spectrum of Hydrocortisone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone and Hydrocortisone RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +150 – +156° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Related substances—Dissolve 20 mg of Hydrocortisone in 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (17:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Dissolve about 20 mg each of Hydrocortisone and Hydrocortisone RS, previously dried and accurately weighed, in 20 mL each of a mixture of chloroform and methanol (9:1), add 10 mL each of the internal standard solution, then add a mixture of chloroform and methanol (9:1) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone to that of the internal standard, respectively.

Amount (mg) of hydrocortisone ($C_{21}H_{30}O_5$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Hydrocortisone RS taken

Internal standard solution—A solution of prednisone in a mixture of chloroform and methanol (9:1) (9 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of chloroform, methanol and acetic acid (100) (1000:20:1).

Flow rate: Adjust so that the retention time of hydrocortisone is about 15 minutes.

System suitability—

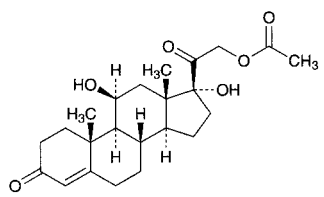
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and hydrocortisone are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Hydrocortisone Acetate

ヒドロコルチゾン酢酸エステル



$C_{23}H_{32}O_6$: 404.50

11β,17,21-Trihydroxypregn-4-ene-3,20-dione 21-acetate

[*50-03-3*]

Hydrocortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Description Hydrocortisone Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in 1,4-dioxane, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Melting point: about 220°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Acetate: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone Acetate in 1 mL of methanol by warming, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.05 g of Hydrocortisone Acetate add 2 mL of potassium hydroxide-ethanol TS, and heat on a water bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(4) Determine the infrared absorption spectra of Hydrocortisone Acetate and Hydrocortisone Acetate RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: both the sample and the RS exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the Reference Standard in ethanol (95), respectively, evaporate to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +158 – +165° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

Purity Related substances—Dissolve 40 mg of Hydrocorti-

sone Acetate in 25 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (160:30:8:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 20 mg each of Hydrocortisone Acetate and Hydrocortisone Acetate RS, previously dried and accurately weighed, in methanol, add exactly 10 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone acetate to that of the internal standard, respectively.

Amount (mg) of hydrocortisone acetate ($C_{23}H_{32}O_6$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Hydrocortisone Acetate RS taken

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust so that the retention time of hydrocortisone acetate is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrocortisone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Hydrocortisone and Diphenhydramine Ointment

ヒドロコルチゾン·ジフェンヒドラミン軟膏

Method of preparation

Hydrocortisone Acetate	5 g
Diphenhydramine	5 g
White Petrolatum	a sufficient quantity
	To make 1000 g

Prepare as directed under Ointments, with the above ingredients.

Description Hydrocortisone and Diphenhydramine Ointment is white to pale yellow in color.

Identification **(1)** To 1 g of Hydrocortisone and Diphenhydramine Ointment add 10 mL of ethanol (95), heat on a water bath for 5 minutes with occasional shaking, cool, and filter. Take 5 mL of the filtrate, distill off the ethanol, and to the residue add 2 mL of sulfuric acid: the solution shows a yellow-green fluorescence immediately and the color of the solution gradually changes through yellow to yellow-brown. Add carefully 10 mL of water to this solution: the color changes to yellow with green fluorescence, and a light yellow, flocculent precipitate is formed (hydrocortisone acetate).

(2) To 1 mL of the filtrate obtained in (1) add 5 mL of potassium hydrogen phthalate buffer solution (pH 4.6) and 2 mL of bromophenol blue TS, and add further 5 mL of chloroform. Shake well, and allow to stand: a yellow color develops in the chloroform layer (diphenhydramine).

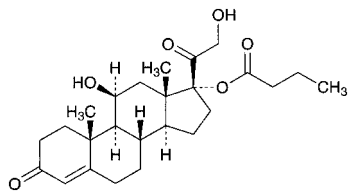
(3) To 1 g of Hydrocortisone and Diphenhydramine Ointment add 5 mL of methanol, warm, and shake. After cooling, separate the methanol layer, and use this layer as the sample solution. Dissolve 10 mg each of hydrocortisone acetate and diphenhydramine in 10 mL each of methanol, and use these solutions as standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with a complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethyl ether (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): two spots from the sample solution show the same *R*f value as the corresponding spots from standard solutions (1) and (2).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Hydrocortisone Butyrate

ヒドロコルチゾン酪酸エステル



$C_{25}H_{36}O_6$: 432.55
11β,17,21-Trihydroxypregn-4-ene-3,20-dione 17-butanoate
[*13609-67-1*]

Hydrocortisone Butyrate, when dried, contains not less than 96.0% and not more than 104.0% of hydrocortisone butyrate ($C_{25}H_{36}O_6$).

Description Hydrocortisone Butyrate occurs as a white powder. It is odorless.

It is freely soluble in tetrahydrofuran, in chloroform and in 1,2-dichloroethane, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 200°C (with decomposition).

Identification **(1)** Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Butyrate: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light (main wavelength: 254 nm). Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone Butyrate in 1 mL of methanol by warming, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 50 mg of Hydrocortisone Butyrate add 2 mL of potassium hydrox-ide-ethanol TS, and heat on a water bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl butyrate is perceptible.

(4) Determine the infrared absorption spectrum of Hydrocortisone Butyrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{25}$: +48 – +52° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Hydrocortisone Butyrate according to method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 25 mg of Hydrocortisone Butyrate in 5 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add tetrahydrofuran to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (470:30:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more than two in number, and not more intense than the spot from the standard solution in color.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

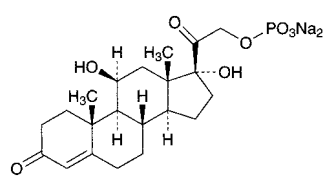
Assay Weigh accurately about 50 mg of Hydrocortisone Butyrate, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, and add ethanol (99.5) to make exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of hydrocortisone butyrate ($C_{25}H_{36}O_6$)
$= A/375 \times 25{,}000$

Containers and storage Containers—Tight containers.

Hydrocortisone Sodium Phosphate

ヒドロコルチゾンリン酸エステルナトリウム



$C_{21}H_{29}Na_2O_8P$: 486.40
11β,17,21-Trihydroxypregn-4-ene-3,20-dione
21-(disodium phosphate)
[*6000-74-4*]

Hydrocortisone Sodium Phosphate contains not less than 96.0% and not more than 102.0% of hydrocortisone sodium phosphate ($C_{21}H_{29}Na_2O_8P$), calculated on the anhydrous basis.

Description Hydrocortisone Sodium Phosphate occurs as a white to light yellow powder.

It is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

It shows crystal polymorphism.

Identification **(1)** To 2 mg of Hydrocortisone Sodium Phosphate add 2 mL of sulfuric acid: a yellowish green fluorescence is exhibited initially, then gradually changes through orange-yellow to dark red. Examine the solution under ultraviolet light (main wavelength: 254 nm): an intense, light green fluorescence is exhibited. To this solution add carefully 10 mL of water: the color changes from yellow to orange-yellow with a light green fluorescence and a yellow-brown, flocculent floating substance is formed.

(2) Determine the infrared absorption spectrum of Hydrocortisone Sodium Phosphate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Hydrocortisone Sodium Phosphate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Phosphate and Hydrocorti-

sone Sodium Phosphate RS in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(3) Moisten 1.0 g of Hydrocortisone Sodium Phosphate with a small quantity of sulfuric acid, and incinerate by gradual heating. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests <*1.09*> for sodium salt and for phosphate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +123 – +131° (1 g calculated on the anhydrous basis, phosphate buffer solution (pH 7.0), 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 100 mL of water: the pH of this solution is between 7.5 and 9.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <*1.03*>—Dissolve 0.30 g of Hydrocortisone Sodium Phosphate in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 100 mL. To 5 mL of this solution add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.600%).

(3) Heavy metals <*1.07*>—Proceed with 0.5 g of Hydrocortisone Sodium Phosphate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Hydrocortisone Sodium Phosphate according to Method 3, and perform the test (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.25 g of Hydrocortisone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution into separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20 ± 1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, at 740 nm of the sample solution and Standard Phosphoric Acid Solution: the amount of free phosphoric acid is not more than 1.0%.

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = 1/M \times A_T/A_S \times 258.0$$

M: Amount (mg) of Hydrocortisone Sodium Phosphate taken, calculated on the anhydrous basis

(6) Free hydrocortisone—Dissolve 25 mg of Hydrocortisone Sodium Phosphate in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh 25 mg of Hydrocortisone RS, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of hydrocortisone in each solution: A_T is not larger than A_S.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrocortisone is not more than 1.0%.

Water <*2.48*> Not more than 5.0% (30 mg, coulometric titration).

Assay Weigh accurately about 20 mg each of Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate RS (previously determine the water <*2.48*> in the same manner as Hydrocortisone Sodium Phosphate), dissolve each in 50 mL of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone phosphate to that of the internal standard, respectively.

$$\text{Amount (mg) of hydrocortisone sodium phosphate } (C_{21}H_{29}Na_2O_8P) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Hydrocortisone Sodium Phosphate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 2.6) and methanol (1:1).

Flow rate: Adjust so that the retention time of hydrocortisone phosphate is about 10 minutes.

System suitability—

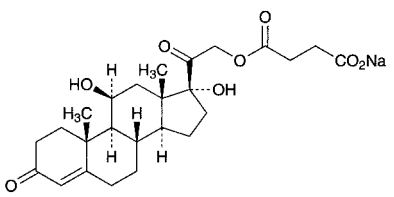
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrocortisone phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone phosphate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Hydrocortisone Sodium Succinate

ヒドロコルチゾンコハク酸エステルナトリウム



$C_{25}H_{33}NaO_8$: 484.51
Monosodium 11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-succinate
[*125-04-2*]

Hydrocortisone Sodium Succinate, calculated on the dried basis, contains not less than 97.0% and not more than 103.0% of hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$).

Description Hydrocortisone Sodium Succinate occurs as white, powder or masses.

It is freely soluble in water, in methanol and in ethanol (95).

It is hygroscopic.

It is gradually colored by light.

It shows crystal polymorphism.

Identification **(1)** Dissolve 0.2 g of Hydrocortisone Sodium Succinate in 20 mL of water, and add 0.5 mL of dilute hydrochloric acid with stirring: a white precipitate is formed. Collect the precipitate, wash it with two 10-mL portions of water, and dry at 105°C for 3 hours. To 3 mg of this dried matter add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Dissolve 10 mg of the dried matter obtained in (1) in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.1 g of the dried matter obtained in (1) add 2 mL of sodium hydroxide TS, and allow to stand for 10 minutes. Filter the solution to remove the precipitate formed, mix the filtrate with 1 mL of dilute hydrochloric acid, filter if necessary, then adjust the solution to a pH of about 6 with diluted ammonia TS (1 in 10), and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Determine the infrared absorption spectrum of the dried matter obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Succinate and Hydrocortisone Succinate RS in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(5) Hydrocortisone Sodium Succinate responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +135 – +145° (0.1 g calculated on the dried basis, ethanol (95), 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Hydrocortisone Sodium Succinate in 5 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 25 mg of Hydrocortisone Sodium Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of hydrocortisone in methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 6 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 3 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution corresponding to the spot obtained from the standard solution (1) is not more intense than the spot from the standard solution (1). Any spot other than the principal spot and the above spot from the sample solution is not more than one, and is not more intense than the spot from the standard solution (2).

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (0.5 g, 105°C, 3 hours).

Assay Weigh accurately about 10 mg of Hydrocortisone Sodium Succinate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Hydrocortisone Succinate RS, previously dried at 105°C for 3 hours, proceed in the same manner as directed for the sample solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

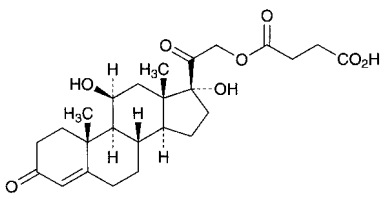
Amount (mg) of hydrocortisone sodium succinate ($C_{25}H_{33}NaO_8$)
$= M_S \times A_T/A_S \times 1.048$

M_S: Amount (mg) of Hydrocortisone Succinate RS taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Hydrocortisone Succinate

ヒドロコルチゾンコハク酸エステル



$C_{25}H_{34}O_8$: 462.53
11β,17,21-Trihydroxypregn-4-ene-3,20-dione
21-(hydrogen succinate)
[*2203-97-6*]

Hydrocortisone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of hydrocortisone succinate ($C_{25}H_{34}O_8$).

Description Hydrocortisone Succinate occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

It shows crystal polymorphism.

Identification **(1)** To 3 mg of Hydrocortisone Succinate add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Hydrocortisone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Succinate and Hydrocortisone Succinate RS in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +147 – +153° (after drying, 0.1 g, ethanol (99.5), 10 mL, 100 nm).

Purity Related substances—Dissolve 25 mg of Hydrocortisone Succinate in exactly 10 mL of methanol, and use this solution as the sample solution. Separately, dissolve 25 mg of hydrocortisone in exactly 10 mL of methanol. Pipet 1 mL of this solution, dilute with methanol to exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 3 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 2.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 50 mg each of Hydrocortisone Succinate and Hydrocortisone Succinate RS, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone succinate to that of the internal standard, respectively.

Amount (mg) of hydrocortisone succinate ($C_{25}H_{34}O_8$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Hydrocortisone Succinate RS taken

Internal standard solution—A solution of butyl parahydroxy benzoate in methanol (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetic acid-sodium acetate buffer solution (pH 4.0) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of hydrocortisone succinate is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, hydrocortisone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

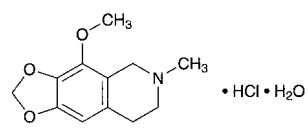
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Hydrocotarnine Hydrochloride Hydrate

ヒドロコタルニン塩酸塩水和物



$C_{12}H_{15}NO_3.HCl.H_2O$: 275.73
4-Methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinoline monohydrochloride monohydrate
[*5985-55-7*, anhydride]

Hydrocotarnine Hydrochloride Hydrate, when dried, contains not less than 98.0% of hydrocotarnine-

hydrochloride ($C_{12}H_{15}NO_3$.HCl: 257.72).

Description Hydrocotarnine Hydrochloride Hydrate occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and slightly soluble in acetic anhydride.

Identification **(1)** Determine the absorption spectrum of a solution of Hydrocotarnine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Hydrocotarnine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Hydrocotarnine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> Dissolve 1.0 g of Hydrocotarnine Hydrochloride Hydrate in 20 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Hydrocotarnine Hydrochloride Hydrate in 10 mL of water: the solution is clear, and when perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank, the absorbance at 400 nm is not more than 0.17.

(2) Heavy metals <*1.07*>—Proceeds with 1.0 g of Hydrocotarnine Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Hydrocotarnine Hydrochloride Hydrate in 10 mL of diluted ethanol (99.5) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (99.5) (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28) (20:20:3:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 7.0% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Hydrocotarnine Hydrochloride Hydrate, previously dried. Dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming. Cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 25.77 mg of $C_{12}H_{15}NO_3$.HCl

Containers and storage Containers—Tight containers.

Hydrogenated Oil

硬化油

Hydrogenated Oil is the fat obtained by hydrogenation of fish oil or of other oils originating from animal or vegetable.

Description Hydrogenated Oil occurs as a white mass or powder and has a characteristic odor and a mild taste.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

The oil obtained by hydrogenation of castor oil is slightly soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Acid value <*1.13*> Not more than 2.0.

Purity **(1)** Moisture and coloration—Hydrogenated Oil (5.0 g), melted by heating on a water bath, forms a clear liquid, from which no water separates. In a 10-mm thick layer of the liquid, it is colorless or slightly yellow.

(2) Alkalinity—To 2.0 g of Hydrogenated Oil add 10 mL of water, melt by heating on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: no color develops.

(3) Chloride—To 1.5 g of Hydrogenated Oil add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the turbidity of the solution does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, then add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

(4) Heavy metals—Heat 2.0 g of Hydrogenated Oil with 5 mL of dilute hydrochloric acid and 10 mL of water on a water bath for 5 minutes with occasional shaking. After cooling, filter, and make 5 mL of the filtrate weakly alkaline with ammonia TS, then add 3 drops of sodium sulfide TS: the solution remains unchanged.

(5) Nickel—Place 5.0 g of Hydrogenated Oil in a quartz or porcelain crucible, heat slightly with caution at the beginning, and, after carbonization, incinerate by strong heating (500 ± 20°C). Cool, add 1 mL of hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 3 mL of dilute hydrochloric acid, and add 7 mL of water. Then add 1 mL of bromine TS and 1 mL of a solution of citric acid monohydrate (1 in 5), make alkaline with 5 mL of ammonia TS, and cool in running water. To this solution add 1 mL of dimethylglyoxime TS, add water to make 20 mL, and use this solution as the test solution. Allow to stand for 5 minutes: the solution has no more color than the following control solution.

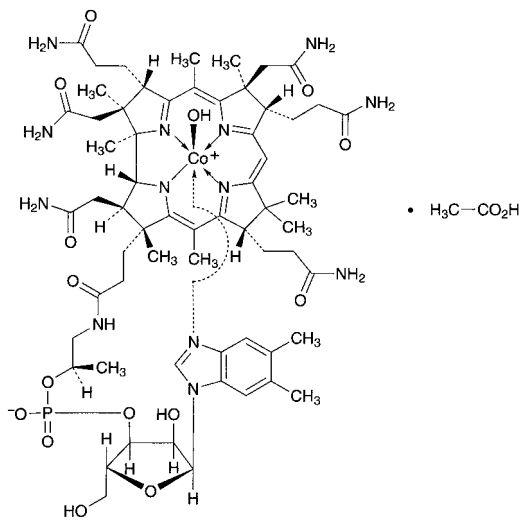
Control solution: Evaporate 1 mL of hydrochloric acid on a water bath to dryness, add 1 mL of Standard Nickel Solution and 3 mL of dilute hydrochloric acid, and add 6 mL of water. Then proceed as directed in the test solution, add water to make 20 mL, and allow to stand for 5 minutes.

Residue on ignition <*2.44*> Not more than 0.1% (5 g).

Containers and storage Containers—Well-closed containers.

Hydroxocobalamin Acetate

ヒドロキソコバラミン酢酸塩



$C_{62}H_{89}CoN_{13}O_{15}P.C_2H_4O_2$: 1406.41
*Co*α-[α-(5,6-Dimethyl-1*H*-benzimidazol-1-yl)]-*Co*β-hydroxocobamide monoacetate
[*13422-51-0*, Hydroxocobalamin]

Hydroxocobalamin Acetate contains not less than 95.0% of hydroxocobalamin acetate ($C_{62}H_{89}CoN_{13}O_{15}P.C_2H_4O_2$), calculated on the dried basis.

Description Hydroxocobalamin Acetate occurs as dark red, crystals or powder. It is odorless.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Hydroxocobalamin Acetate in acetic acid-sodium acetate buffer solution (pH 4.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Hydroxocobalamin Acetate with 50 mg of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until the solution develops a light red. Then add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color develops immediately. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Add 0.5 mL of ethanol (99.5) and 1 mL of sulfuric acid to 20 mg of Hydroxocobalamin Acetate, and heat the mixture: the odor of ethyl acetate is perceptible.

Purity Cyanocobalamin and colored impurities—Dissolve 50 mg of Hydroxocobalamin Acetate in exactly 5 mL each of acetic acid-sodium acetate buffer solution (pH 5.0), in two tubes. To one tube add 0.15 mL of potassium thiocyanate TS, allow to stand for 30 minutes, and use this solution as the sample solution (1). To the other tube add 0.10 mL of potassium cyanide TS, allow to stand for 30 minutes, and use this solution as the sample solution (2). Separately, dissolve 3.0 mg of Cyanocobalamin RS in exactly 10 mL of acetic acid-sodium acetate buffer solution (pH 5.0) and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution (1), (2) and standard solution 25 mm in length along the starting line, 10 mm apart from each other, on a plate of silica gel for thin-layer chromatography. Develop the plate for 18 hours with 2-butanol saturated with water, while supporting the plate at an angle of about 15° to a horizontal plane, and air-dry the plate: the spot from the sample solution (1) corresponding to that from the standard solution is not more intense than the spot from the standard solution, and the spots other than the principal spot from the sample solution (2) are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 12% (50 mg, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 6 hours).

Assay Weigh accurately about 20 mg of Hydroxocobalamin Acetate, and dissolve in acetic acid-sodium acetate buffer solution (pH 5.0) to make exactly 50 mL. Pipet 2 mL of this solution into a 50-mL volumetric flask, add 1 mL of a solution of potassium cyanide (1 in 1000), and allow to stand for 30 minutes at ordinary temperature. Add acetic acid-sodium acetate buffer solution (pH 5.0) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cyanocobalamin RS (separately determine the loss on drying <*2.41*> under the same conditions as Cyanocobalamin), and dissolve in water to make exactly 50 mL. To 2 mL of this solution, exactly measured, add acetic acid-sodium acetate buffer solution (pH 5.0) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 361 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of hydroxocobalamin acetate ($C_{62}H_{89}CoN_{13}O_{15}P.C_2H_4O_2$)
$= M_S \times A_T/A_S \times 1.038$

M_S: Amount (mg) of Cyanocobalamin RS taken, calculated on the dried basis

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Hydroxypropylcellulose

Cellulose, 2-hydroxypropyl ether

ヒドロキシプロピルセルロース

[*9004-64-2*]

This monograph is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Hydroxypropylcellulose is partially *O*-(2-hydroxypropylated)cellulose.

Hydroxypropylcellulose contains not less than 53.4% and not more than 80.5% of hydroxypropoxy group (-OC_3H_6OH: 75.09), calculated on the dried basis.

It may contain silicon dioxide as anti-caking agent.

◆The label states the addition in the case where silicon dioxide is added as anti-caking agent.◆

◆**Description** Hydroxypropylcellulose occurs as a white to yellowish white powder.

It forms a viscous liquid upon addition of water or ethanol (95).◆

Identification **(1)** Dissolve 1 g of Hydroxypropylcellulose in 100 mL of water, transfer 1 mL of the solution to a glass plate, and allow the water to evaporate: a thin film is formed.

(2) Determine the infrared absorption spectrum of Hdroxypropylcellulose as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If there are an absorption at about 1719 cm^{-1}, disregard the absorption.

pH <*2.54*> Disperse evenly 1.0 g of Hydroxypropylcellulose in 100 mL of freshly boiled water, and allow to cool the mixture while stirring with a magnetic stirrer the pH of the solution is between 5.0 and 8.0.

Purity

◆**(1)** Heavy metals<*1.07*>—Proceed with 1.0 g of Hydroxypropylcellulose according to Method 2 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

(2) Silicon dioxide—Apply to Hydroxypropylcellulose, if the addition of silicon dioxide is stated on the label and if more than 0.2% residue is found in the Residue on ignition test. Weigh accurately the crucible containing the residue tested in the Residue on ignition of Hydroxypropylcellulose (*a* (g)). Moisten the residue with water, and add 5 mL of hydrochloric acid, in small portions. Evaporate it on a steam bath to dryness and cool. Add 5 mL of hydrofluoric acid and 0.5 mL of sulfuric acid, and evaporate to dryness. Slowly increase the temperature until all the acids have been volatilized, and ignite at 1000 ± 25°C. Cool the crucible in a desiccator, and weigh (*b* (g)). Calculate the amount of silicon dioxide by the following equation: not more than 0.6%.

$$\text{Amount (\%) of silicon dioxide } (SiO_2) = (a - b)/M \times 100$$

M: Amount (g) of Hydroxypropylcellulose used for residue on ignition test

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.8% (1 g, platinum crucible).

Assay Weigh accurately about 30 mg of Hydroxypropylcellulose, transfer to a reaction vial, add exactly 60 mg of adipic acid, 2 mL of the internal standard solution and 1 mL of hydriodic acid, stopper the vial tightly, and weigh accurately. Place the vial in an oven or heat by a suitable heater with continuous stirring, maintaining the internal temperature of 115 ± 2°C for 70 minutes. Allow the vial to cool and weigh accurately. If the difference of the mass between before heating and after heating is more than 10 mg, prepare a new test solution. If the difference of the mass between before heating and after heating is not more than 10 mg, after phase separation by allowing the vial to stand, pierce through the septum of the vial with a cooled syringe, and withdraw a sufficient volume of the upper phase as test solution. Separately, place exactly 60 mg of adipic acid, 2 mL of internal standard solution and 1 mL of hydriodic acid in an another reaction vial, stopper tightly, and weigh accurately. Inject 25 μL of isopropyl iodide for assay through the septum, and again weigh accurately. Shake the vial well, and after phase separation by allowing the vial to stand, pierce through the septum of the vial with a cooled syringe, and withdraw a sufficient volume of the upper phase as the standard solution. Perform the test as directed under Gas Chromatography <*2.02*> with 2 μL each of the sample solution and standard solution according to the following conditions, calculate the ratios, Q_T and Q_S, of the peak area of isopropyl iodide to that of the internal standard.

$$\text{Amount (\%) of hydroxypropoxy group } (C_3H_7O_2) = (1.15 \times Q_T \times F \times 75.1 \times 100)/(M_T \times 170.0)$$

$$F = (M_S \times C)/(Q_S \times 100)$$

M_T: Amount (mg) of Hydroxypropylcellulose taken, calculated on the dried basis
M_S: Amount (mg) of isopropyl iodide for assay taken
F: Response factor
C: Amount (%) of isopropyl iodide for assay
75.1: Molecular mass of hydroxypropoxy group
170.0: Molecular mass of isopropyl iodide
1.15: Correction factor

Internal standard solution—A solution of methylcyclohexan in o-xylene (1 in 50).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.53 mm in diameter and 30 m in length, coated on the inner wall with methylsilicone polymer for gas chromatography in 3 μm thickness.

Column Temperature: 40°C for 3 minutes, then rise up to 100°C at the rate of 10°C per minute, and then rise up to 250°C at the rate of 50°C per minutes, and maintain at a constant temperature of 250°C for 3 minutes.

Injection port temperature: A constant temperature of about 180°C.

Detector temperature: A constant temperature of about 280°C.

Carrier gas: Helium.

Flow rate: 52 cm per second.

Split ratio: 1:50.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, isopropyl iodide and the internal standard are eluted in this order, with the relative retention time of isopropyl iodide to the internal standard being about 0.8, and with the resolution between the peaks of isopropyl iodide and the internal standard being not less than 2.0.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the response factor F is not more than 2.0%.

◆**Containers and storage** Containers—Well-closed containers.◆

Low Substituted Hydroxypropylcellulose

低置換度ヒドロキシプロピルセルロース

[*9004-64-2*, Hydroxypropylcellulose]

Low Substituted Hydroxypropylcellulose is a low substituted hydroxypropyl ether of cellulose.

Low Substituted Hydroxypropylcellulose, when dried, contains not less than 5.0% and not more than 16.0% of hydroxypropoxy group ($-OC_3H_6OH$: 75.09).

Description Low Substituted Hydroxypropylcellulose occurs as a white to yellowish white, powder or granules. It is odorless or has a slight, characteristic odor. It is tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in a solution of sodium hydroxide (1 in 10), and produces a viscous solution.

It swells in water, in sodium carbonate TS and in 2 mol/L hydrochloric acid TS.

Identification **(1)** To 20 mg of Low Substituted Hydroxypropylcellulose add 2 mL of water, shake, and produce a turbid solution. Add 1 mL of anthrone TS gently: a blue to blue-green color develops at the zone of contact.

(2) To 0.1 g of Low Substituted Hydroxypropylcellulose add 10 mL of water, stir and produce a turbid solution. Add 1 g of sodium hydroxide, shake until it becomes homogeneous, and use this solution as the sample solution. To 0.1 mL of the sample solution add 9 mL of diluted sulfuric acid (9 in 10), shake well, heat in a water bath for exactly 3 minutes, immediately cool in an ice bath, add carefully 0.6 mL of ninhydrin TS, shake well, and allow to stand at 25°C: a red color develops at first, and it changes to purple within 100 minutes.

(3) To 5 mL of the sample solution obtained in (2) add 10 mL of a mixture of acetone and methanol (4:1), and shake: a white, flocculent precipitate is produced.

pH <*2.54*> To 1.0 g of Low Substituted Hydroxypropylcellulose add 100 mL of freshly boiled and cooled water, and shake: the pH of the solution is between 5.0 and 7.5.

Purity **(1)** Chloride <*1.03*>—To 0.5 g of Low Substituted Hydroxypropylcellulose add 30 mL of hot water, stir well, heat on a water bath for 10 minutes, and filter the supernatant liquid by decantation while being hot. Wash the residue thoroughly with 50 mL of hot water, combine the washings with the filtrate, and add water to make 100 mL after cooling. To 5 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.355%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Low Substituted Hydroxypropylcellulose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Low Substituted Hydroxypropylcellulose, according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 6.0% (1 g, 105°C, 1 hour).

Residue on ignition <*2.44*> Not more than 1.0% (1 g).

Assay (i) Apparatus—Reaction flask: A 5-mL screw-cap pressure-tight glass bottle, having an inverted conical bottom inside, 20 mm in outside diameter, 50 mm in height up to the neck, 2 mL in capacity up to a height of about 30 mm, equipped with a pressure-tight septum of heat-resisting resin and also with an inside stopper or sealer of fluoroplastic.

Heater: A square-shaped aluminum block 60 to 80 mm thick, having holes 20.6 mm in diameter and 32 mm in depth, capable of maintaining the inside temperature within ±1°C.

(ii) Procedure—Weigh accurately about 65 mg of Low Substituted Hydroxypropylcellulose, previously dried, transfer to the reaction flask, add 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydriodic acid, stopper the flask tightly, and weigh accurately. Shake the flask for 30 seconds, heat at 150°C on the heater for 30 minutes with repeated shaking at 5-minute intervals, and continue heating for an additional 30 minutes. Allow the flask to cool, and again weigh accurately. If the mass loss is less than 10 mg, use the upper layer of the mixture as the sample solution. Separately, take 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydriodic acid in another reaction flask, stopper tightly, and weigh accurately. Add 15 μL of isopropyl iodide for assay, and again weigh accurately. Shake the reaction flask for 30 seconds, and use the upper layer of the content as the standard solution. Perform the test as directed under Gas Chromatography <*2.02*> with 2 μL each of the sample solution and standard solution according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of isopropyl iodide to that of the internal standard.

$$\text{Amount (\%) of hydroxypropoxy group } (C_3H_7O_2) = M_S/M_T \times Q_T/Q_S \times 44.17$$

M_S: Amount (mg) of isopropyl iodide for assay taken
M_T: amount (mg) of Low Substituted Hydroxypropylcellulose taken

Internal standard solution—A solution of *n*-octane in *o*-xylene (1 in 50).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with methyl silicone polymer for gas chromatography at the ratio of 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium (for thermal-conductivity detector); Helium or Nitrogen (for hydrogen flame-ionization detector).

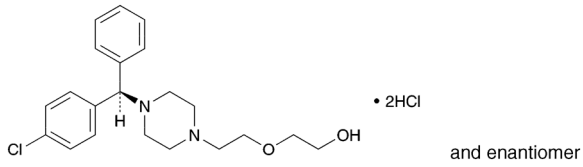
Flow rate: Adjust so that the retention time of the internal standard is about 10 minutes.

Selection of column: Proceed with 1 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of isopropyl iodide and the internal standard in this order.

Containers and storage Containers—Tight containers.

Hydroxyzine Hydrochloride

ヒドロキシジン塩酸塩



and enantiomer

$C_{21}H_{27}ClN_2O_2.2HCl$: 447.83
2-(2-{4-[(*RS*)-(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)ethanol dihydrochloride
[*2192-20-3*]

Hydroxyzine Hydrochloride, when dried, contains not less than 98.5% of hydroxyzine hydrochloride ($C_{21}H_{27}ClN_2O_2.2HCl$).

Description Hydroxyzine Hydrochloride occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 200°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Hydroxyzine Hydrochloride (1 in 100) add 2 to 3 drops of ammonium thiocyanate-cobalt (II) nitrate TS: a blue precipitate is formed.

(2) Determine the absorption spectrum of a solution of Hydroxyzine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Hydroxyzine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> Dissolve 1.0 g of Hydroxyzine Hydrochloride in 20 mL of water: the pH of this solution is between 1.3 and 2.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Hydroxyzine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Hydroxyzine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Hydroxyzine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and ammonia solution (28) (150:95:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

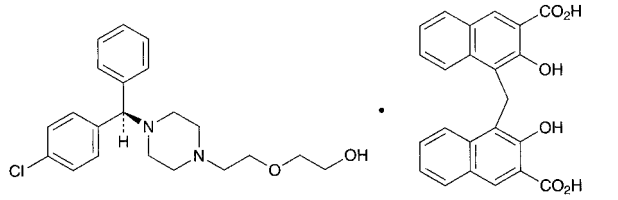
Assay Weigh accurately about 0.1 g of Hydroxyzine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 22.39 mg of $C_{21}H_{27}ClN_2O_2.2HCl$

Containers and storage Containers—Tight containers.

Hydroxyzine Pamoate

ヒドロキシジンパモ酸塩



and enantiomer

$C_{21}H_{27}ClN_2O_2.C_{23}H_{16}O_6$: 763.27
2-(2-{4-[(*RS*)-(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)ethanol mono[4,4′-methylenebis(3-hydroxy-2-naphthoate)]
[*10246-75-0*]

Hydroxyzine Pamoate contains not less than 98.0% of hydroxyzine pamoate ($C_{21}H_{27}ClN_2O_2.C_{23}H_{16}O_6$), calculated on the anhydrous basis.

Description Hydroxyzine Pamoate occurs as a light yellow crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in *N*,*N*-dimethylformamide, slightly soluble in acetone, and practically insoluble in water, in methanol, in ethanol (95) and in diethyl ether.

Identification **(1)** To 0.1 g of Hydroxyzine Pamoate add 25 mL of sodium hydroxide TS, and shake well. Extract with 20 mL of chloroform, and use the chloroform layer as the sample solution. Use the water layer for test (4). To 5 mL of the sample solution add 2 mL of ammonium thiocyanate-cobalt (II) nitrate TS, shake well, and allow to stand: a blue color is produced in the chloroform layer.

(2) Evaporate 2 mL of the sample solution obtained in (1) on a water bath to dryness, and dissolve the residue in 0.1 mol/L hydrochloric acid TS to make 500 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Hydroxyzine Pamoate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(4) To 1 mL of the water layer obtained in (1), add 2 mL of 1 mol/L hydrochloric acid TS: a yellow precipitate is produced. Collect the precipitate, dissolve the precipitate in 5 mL of methanol, and add 1 drop of iron (III) chloride TS: a green color is produced.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Hydroxyzine Pamoate in 10 mL of *N*,*N*-dimethylformamide: the solution is clear, and shows a slightly greenish,

light yellow-brown color.

(2) Chloride <*1.03*>—To 0.3 g of Hydroxyzine Pamoate add 6 mL of dilute nitric acid and 10 mL of water, shake for 5 minutes, and filter. Wash the residue with two 10-mL portions of water, combine the washings with the filtrate, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.095%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Hydroxyzine Pamoate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Hydroxyzine Pamoate according to Method 3, and perform the test (not more than 1 ppm).

(5) Related substances—Dissolve 0.40 g of Hydroxyzine Pamoate in 10 mL of a mixture of sodium hydroxide TS and acetone (1:1), and use the solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of sodium hydroxide TS and acetone (1:1) to make exactly 20 mL. Pipet 5 mL of this solution, add a mixture of sodium hydroxide TS and acetone (1:1) to make exactly 50 mL, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and ammonia TS (150:95:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots other than hydroxyzine and pamoic acid obtained from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 3.0% (1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.5% (1 g).

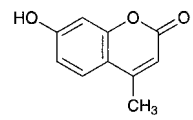
Assay Weigh accurately about 0.6 g of Hydroxyzine Pamoate, add 25 mL of sodium hydroxide TS, shake well, and extract with six 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, and evaporate the combined chloroform extracts on a water bath to about 30 mL. Add 30 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 38.16 mg of $C_{21}H_{27}ClN_2O_2.C_{23}H_{16}O_6$

Containers and storage Containers—Tight containers.

Hymecromone

ヒメクロモン



$C_{10}H_8O_3$: 176.17
7-Hydroxy-4-methylchromen-2-one
[*90-33-5*]

Hymecromone, when dried, contains not less than 98.0% of hymecromone ($C_{10}H_8O_3$).

Description Hymecromone occurs as white, crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in ethanol (95), in ethanol (99.5) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

Identification **(1)** Dissolve 2 mg of Hymecromone in 5 mL of ammonia-ammonium chloride buffer solution (pH 11.0): the solution shows an intense blue-purple fluorescence.

(2) Dissolve 25 mg of Hymecromone in 5 mL of diluted ethanol (95) (1 in 2), and add 1 drop of iron (III) chloride TS: initially a blackish brown color develops, and when allowed to stand the color changes to yellow-brown.

(3) Determine the absorption spectrum of a solution of Himecromone in ethanol (99.5) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Himecromone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 187 – 191°C

Purity **(1)** Chloride <*1.03*>—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2:1), and add 6 mL of dilute nitric acid and a mixture of acetone and water (2:1) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and a mixture of acetone and water (2:1) to make 50 mL (not more than 0.011%).

(2) Sulfate <*1.14*>—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2:1), and add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2:1) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2:1) to make 50 mL (not more than 0.024%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Hymecromone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Hymecromone according to Method 3, and perform the

test (not more than 2 ppm).

(5) Related substances—Dissolve 80 mg of Hymecromone in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (10:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Hymecromone, previously dried, dissolve in 90 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution prepared by adding 14 mL of water to 90 mL of *N*,*N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 17.62 mg of $C_{10}H_8O_3$

Containers and storage Containers—Tight containers.

Hypromellose

Hydroxypropylmethylcellulose

ヒプロメロース

[*9004-65-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Hypromellose is a methyl and hydroxypropyl mixed ether of cellulose.

There are four substitution types of Hypromellose, 1828, 2208, 2906 and 2910. They contain methoxy ($-OCH_3$: 31.03) and hydroxypropoxy ($-OC_3H_6OH$: 75.09) groups conforming to the limits for the types of Hypromellose shown in the table below, calculated on the dried basis.

The viscosity is shown in millipascal second (mPa·s) on the label, together with the substitution type.

Substitution Type	Methoxy Group (%)		Hydroxypropoxy Group (%)	
	Min.	Max.	Min.	Max.
1828	16.5	20.0	23.0	32.0
2208	19.0	24.0	4.0	12.0
2906	27.0	30.0	4.0	7.5
2910	28.0	30.0	7.0	12.0

◆**Description** Hypromellose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

It swells with water and becomes a clear or slightly turbid, viscous solution.◆

Identification **(1)** Disperse evenly 1.0 g of Hypromellose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

(2) Add 1.0 g of Hypromellose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 10°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

(3) To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of diluted sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water. Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color first, then changes to purple color within 100 minutes.

(4) Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

(5) Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

Viscosity <*2.53*> (i) Method I: Apply to Hypromellose having a labeled viscosity of less than 600 mPa·s. Put an exact amount of Hypromellose, equivalent to 4.000 g calculated on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350-to 450-revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 10°C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Hypromellose having a labeled viscosity of not less than 600 mPa·s. Put an exact amount of Hypromellose, equivalent to 10.00 g calculated on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model.

Rotor No., rotation frequency, and calculation multiplier: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)				Rotor No.	Rotation frequency /min	Calculation multiplier
Not less than 600 and less than			1400	3	60	20
〃	1400	〃	3500	3	12	100
〃	3500	〃	9500	4	60	100
〃	9500	〃	99,500	4	6	1000
〃	99,500			4	3	2000

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for at least 2 minutes. Repeat this procedure more two times, and average three observed values.

pH ⟨*2.54*⟩ The pH of the sample solution obtained in the Viscosity, measured after 5 minutes immersing the electrode in the sample solution, is between 5.0 and 8.0.

◆**Purity** Heavy metals—Put 1.0 g of Hypromellose in a 100-mL Kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until to use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution (pH 3.5) and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).◆

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition ⟨*2.44*⟩ Not more than 1.5% (1 g).

Assay (i) Apparatus—Reaction vial: A 5-mL pressure-tight serum vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A square-shaped aluminum block, having holes 20 mm in diameter and 32 mm in depth, adopted to the reaction vial. Capable of stirring the content of the reaction vial by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Hypromellose, transfer to the reaction vial, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydriodic acid, stopper the vial immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the vial content is 130 ± 2°C. In the case when the magnetic stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the vial to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydriodic acid in a reaction vial, stopper the vial immediately, and weigh accurately. Add 45 μL of iodomethane for assay and 15 to 22 μL of isopropyl iodide for assay through the septum using micro-syringe with weighing accurately every time, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of iodomethane and isopropyl iodide to that of the internal standard obtained from the sample solution, and Q_{Sa} and Q_{Sb}, of the peak area of iodomethane and isopropyl iodide to that of the internal standard from the standard solution.

$$\text{Content (\%) of methoxy group } (-CH_3O) = Q_{Ta}/Q_{Sa} \times M_{Sa}/M \times 21.86$$

$$\text{Content (\%) of hydroxypropoxy group } (-C_3H_7O_2) = Q_{Tb}/Q_{Sb} \times M_{Sb}/M \times 44.17$$

M_{Sa}: Amount (mg) of iodomethane for assay taken
M_{Sb}: Amount (mg) of isopropyl iodide for assay taken
M: Amount (mg) of Hypromellose taken, calculated on the dried basis

Internal standard solution—A solution of *n*-octane in *o*-xylene (3 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen flame-ionization detector.

Flow rate: Adjust so that the retention time of the internal standard is about 10 minutes.

System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane, isopropyl iodide and the internal standard are eluted in this order, with complete separation of these peaks.

◆**Containers and storage** Containers—Well-closed containers.◆

Hypromellose Acetate Succinate

ヒプロメロース酢酸エステルコハク酸エステル

[*71138-97-1*]

Hypromellose Acetate Succinate is an acetic acid and monosuccinic acid mixed ester of hypromellose.

It contains not less than 12.0% and not more than 28.0% of methoxy group (-OCH_3: 31.03), not less than 4.0 and not more than 23.0% of hydroxypropoxy group (-OC_3H_6OH: 75.09), not less than 2.0% and not more than 16.0% of acetyl group (-$COCH_3$: 43.04), and not less than 4.0% and not more than 28.0% of succinyl group (-COC_2H_4COOH: 101.08), calculated on the dried basis.

Its viscosity is expressed in millipascal second (mPa·s).

Description Hypromellose Acetate Succinate occurs as a white to yellowish white, powder or granules.

It is practically insoluble in water and in ethanol (99.5).

It dissolves in sodium hydroxide TS.

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Hypromellose Acetate Succinate as directed in the ATR method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2840 cm^{-1}, 1737 cm^{-1}, 1371 cm^{-1}, 1231 cm^{-1} and 1049 cm^{-1}.

Viscosity <*2.53*> To 2.00 g of Hypromellose Acetate Succinate, previously dried, add dilute sodium hydroxide TS to make 100.0 g, stopper tightly, and dissolve by shaking for 30 minutes. Perform the test with this solution at 20°C according to Method 1: 80 – 120% of the labeled viscosity.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Hypromellose Acetate Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Free acetic acid and free succinic acid—Weigh accurately about 0.1 g of Hypromellose Acetate Succinate, add exactly 4 mL of 0.02 mol/L phosphate buffer solution (pH 7.5), stopper tightly, and stir for 2 hours. Then add exactly 4 mL of diluted phosphoric acid (1 in 500), turn the test tube upside down several times, centrifuge, and use the supernatant liquid as the sample solution. Separately, place 20 mL of water in a 100-mL volumetric flask, weigh the mass of the flask accurately, then add 2.0 mL of acetic acid (100), weigh the mass of the flask to calculate the accurate mass of added acetic acid, and dilute with water to volume. Pipet 6 mL of this solution, add water to make exactly 100 mL, and use this solution as the acetic acid stock solution. Separately, weigh accurately about 0.13 g of succinic acid, dissolve in water to make exactly 100 mL, and use this solution as the succinic acid stock solution. Pipet exactly 4 mL each of the acetic acid stock solution and the succinic acid stock solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas of acetic acid and succinic acid of both solutions, A_{TA}, A_{TS} and A_{SA}, A_{SS}, and calculate the amount of free acetic acid and free succinic acid by the following expressions: the total amount is not more than 1.0%.

Amount (%) of free acetic acid ($C_2H_4O_2$)
$= M_{SA}/M_T \times A_{TA}/A_{SA} \times 48/625$

Amount (%) of free succinic acid ($C_4H_6O_4$)
$= M_{SS}/M_T \times A_{TS}/A_{SS} \times 32/25$

M_{SA}: Amount (mg) of acetic acid (100) taken
M_{SS}: Amount (mg) of succinic acid taken
M_T: Amount (mg) of Hypromellose Acetate Succinate taken, calculated on the dried basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (1).

Test for required detectability: To 3 mL of the standard solution add the mobile phase to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak areas of acetic acid and succinic acid obtained with 10 μL of this solution are equivalent to 7 to 13% of corresponding those obtained with 10 μL of the solution for system suitability test.

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay **(1)** Acetyl group and succinyl group—Weigh accurately about 30 mg of Hypromellose Acetate Succinate, add exactly 10 mL of sodium hydroxide TS, stopper tightly, and stir for 4 hours. Add exactly 10 mL of diluted phosphoric acid (17 in 200), turn the test tube upside down several times, and filter the solution through a membrane filter with a pore size 0.22 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, place 20 mL of water in a 100-mL volumetric flask, weigh the mass of the flask accurately, then add 2.0 mL of acetic acid (100), weigh the mass of the flask to calculate the accurate mass of added acetic acid, and dilute with water to volume. Pipet 6 mL of this solution, add water to make exactly 100 mL, and use this solution as the acetic acid stock solution. Separately, weigh accurately about 0.13 g of succinic acid, dissolve in water to make exactly 100 mL, and use this solution as the succinic acid stock solution. Pipet 4 mL each of the acetic acid stock solution and the succinic acid stock solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TA}, A_{TS} and A_{SA}, A_{SS}, of acetic acid and succinic acid in each solution.

Amount (%) of acetyl group (C_2H_3O)
$= (M_{SA}/M_T \times A_{TA}/A_{SA} \times 24/125 - A_{free}) \times 0.717$

Amount (%) of succinyl group ($C_4H_5O_3$)
$= (M_{SS}/M_T \times A_{TS}/A_{SS} \times 16/5 - S_{free}) \times 0.856$

M_{SA}: Amount (mg) of acetic acid (100) taken
M_{SS}: Amount (mg) of succinic acid taken
M_T: Amount (mg) of Hypromellose Acetate Succinate taken, calculated on the dried basis
A_{free}: Amount (%) of free acetic acid obtained in the Purity (2)
S_{free}: Amount (%) of free succinic acid obtained in the

Purity (2)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: 0.02 mol/L potassium dihydrogen phosphate TS, adjusted to pH 2.8 with phosphoric acid.

Flow rate: Adjust so that the retention time of succinic acid is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, acetic acid and succinic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetic acid and succinic acid is not more than 2.0%.

(2) Methoxy group and hydroxypropoxy group

(i) Apparatus—Reaction bottle: A 5 mL pressure-tight glass vial, having 20 mm in outside diameter, 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A square-shaped aluminum block, having holes 20 mm in inside diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the bottle by means of magnetic stirrer or reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Hypromellose Acetate Succinate, place in the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydriodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is 130 ± 2°C. In a case where the magnetic stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5 minute intervals by hand, and continue heating for additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydriodic acid in a reaction bottle, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay and 15 to 22 μL of isopropyl iodide for assay through the septum using a micro-syringe with weighing accurately every time, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios of the peak areas of iodomethane and isopropyl iodide to the peak area of the internal standard, Q_{Ta}, Q_{Tb} and Q_{Sa}, Q_{Sb}.

Amount (%) of methoxy group (CH_3O)
$= M_{Sa}/M_T \times Q_{Ta}/Q_{Sa} \times 21.86$

Amount (%) of hydroxypropoxy group ($C_3H_7O_2$)
$= M_{Sb}/M_T \times Q_{Tb}/Q_{Sb} \times 44.17$

M_{Sa}: Amount (mg) of iodomethane for assay taken

M_{Sb}: Amount (mg) of isopropyl iodide for assay taken

M_T: Amount (mg) of Hypromellose Acetate Succinate taken, calculated on the dried basis

Internal standard solution—A solution of *n*-octane in *o*-xylene (3 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass tube 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 120 to 150 μm in diameter coated with methyl silicon polymer for gas chromatography in 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for the thermal conductivity detector, or Helium or Nitrogen for the hydrogen flame-ionization detector.

Flow rate: Adjust so that the retention time of the internal standard is about 10 minutes.

System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane, isopropyl iodide and the internal standard are eluted in this order with the resolution between each peak being not less than 5.

System repeatability: When the test is repeated 6 times with 1 – 2 μL of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of iodomethane and isopropyl iodide to that of the internal standard are not more than 2.0%, respectively.

Containers and storage Containers—Tight containers.

Hypromellose Phthalate

ヒプロメロースフタル酸エステル

[*9050-31-1*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Hypromellose Phthalate is a monophthalic acid ester of hypromellose.

It contains methoxy group ($-OCH_3$: 31.03), hydroxypropoxy group ($-OCH_2CHOHCH_3$: 75.09), and carboxybenzoyl group ($-COC_6H_4COOH$: 149.12).

It contains not less than 21.0% and not more than 35.0% of carboxybenzoyl group, calculated on the anhydrous basis.

◆Its substitution type and its viscosity in millipascal second (mPa·s) are shown on the label.

Substitution Type	Carboxybenzoyl group (%) Min.	Max.
200731	27.0	35.0
220824	21.0	27.0

◆

◆**Description** Hypromellose Phthalate occurs as white, powder or granules.

It is practically insoluble in water, in acetonitrile and in ethanol (99.5).

It becomes a viscous liquid when a mixture of methanol and dichloromethane (1:1) or a mixture of ethanol (99.5) and acetone (1:1) is added.

It dissolves in sodium hydroxide TS.◆

◆**Identification** Determine the infrared absorption spectrum of Hypromellose Phthalate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Viscosity ⟨*2.53*⟩ To 10 g of Hypromellose Phthalate, previously dried at 105°C for 1 hour, add 90 g of a mixture of methanol and dichloromethane in equal mass ratio, and stir to dissolve. Determine the viscosity at 20 ± 0.1°C as directed in Method 1 under Viscosity Determination: the viscosity is not less than 80% and not more than 120% of the labeled unit.

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Hypromellose Phthalate in 40 mL of 0.2 mol/L sodium hydroxide VS, add 1 drop of phenolphthalein TS, and add dilute nitric acid dropwise with vigorous stirring until the red color is discharged. Further add 20 mL of dilute nitric acid with stirring. Heat on a water bath with stirring until the gelatinous precipitate formed turns to granular particles. After cooling, centrifuge, and take off the supernatant liquid. Wash the precipitate with three 20-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, add water to make 200 mL, and filter. Perform the test with 50 mL of the filtrate. Control solution: To 0.50 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of 0.2 mol/L sodium hydroxide VS and 7 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.07%).

◆**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Hypromellose Phthalate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(3) Phthalic acid—Weigh accurately about 0.2 g of Hypromellose Phthalate, add about 50 mL of acetonitrile to dissolve partially with the aid of ultrasonic waves, add 10 mL of water, and dissolve further with the ultrasonic waves. After cooling, add acetonitrile to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 12.5 mg of phthalic acid, dissolve in about 125 mL of acetonitrile by mixing, add 25 mL of water, then add acetonitrile to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of phthalic acid in each solution: amount of phthalic acid ($C_8H_6O_4$: 166.13) is not more than 1.0%.

$$\text{Amount (\%) of phthalic acid} = M_S/M_T \times A_T/A_S \times 40$$

M_S: Amount (mg) of phthalic acid taken
M_T: Amount (mg) of Hypromellose Phthalate taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of 0.1% trifluoroacetic acid and acetonitrile (9:1).

Flow rate: About 2.0 mL per minute.

System suitability—

◆System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of phthalic acid are not less than 2500 and not more than 1.5, respectively.◆

System repeatability: When repeat the test 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of phthalic acid is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 5.0% (1 g, volumetric titration, direct titration, using a mixture of ethanol (99.5) and dichloromethane (3:2) instead of methanol for water determination).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 1 g of Hypromellose Phthalate, dissolve in 50 mL of a mixture of ethanol (95), acetone and water (2:2:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Amount (\%) of carboxybenzoyl group } (C_8H_5O_3) = \{(0.01 \times 149.1 \times V)/M\} - \{(2 \times 149.1 \times P)/166.1\}$$

P: Amount (%) of phthalic acid obtained in the Purity (3)
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed
M: Amount (g) of Hypromellose Phthalate taken, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

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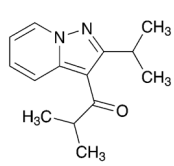


$C_{14}H_{18}N_2O$: 230.31
1-[2-(1-Methylethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-methylpropan-1-one
[*50847-11-5*]

Ibudilast, when dried, contains not less than 98.5% and not more than 101.0% of ibudilast ($C_{14}H_{18}N_2O$).

Description Ibudilast occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5) and in acetic anhydride, and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Ibudilast in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same

wavelengths.

(2) Determine the infrared absorption spectrum of Ibudilast as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 54 – 58°C

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Ibudilast according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Ibudilast in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than ibudilast obtained from the sample solution is not larger than the peak area of ibudilast obtained from the standard solution, and the total area of the peaks other than ibudilast is not larger than 3 times the peak area of ibudilast from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column 2.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane and ethyl acetate (50:1).

Flow rate: Adjust so that the retention time of ibudilast is about 9 minutes.

Time span of measurement: About 4 times as long as the retention time of ibudilast, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ibudilast obtained with 10 μL of this solution is equivalent to 40 to 60% of that obtained with 10 μL of the standard solution.

System performance: To 5 mL of the sample solution add the mobile phase to make 50 mL. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ibudilast are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ibudilast is not more than 3.0%.

Loss on drying <2.41> Not more than 0.3% (1 g, in vacuum, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

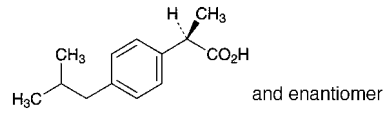
Assay Weigh accurately about 0.2 g of Ibudilast, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.03 mg of $C_{14}H_{18}N_2O$

Containers and storage Containers—Tight containers.

Ibuprofen

イブプロフェン



$C_{13}H_{18}O_2$: 206.28

(2*RS*)-2-[4-(2-Methylpropyl)phenyl]propanoic acid

[*15687-27-1*]

Ibuprofen, when dried, contains not less than 98.5% of ibuprofen ($C_{13}H_{18}O_2$).

Description Ibuprofen occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

Identification **(1)** Determine the absorption spectrum of a solution of Ibuprofen in dilute sodium hydroxide TS (3 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ibuprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 75 – 77°C

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of Ibuprofen according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ibuprofen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.50 g of Ibuprofen in 5 mL of acetone , and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

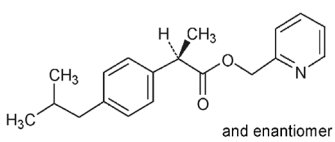
Assay Weigh accurately about 0.5 g of Ibuprofen, previously dried, dissolve in 50 mL of ethanol (95), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 20.63 mg of $C_{13}H_{18}O_2$

Containers and storage Containers—Well-closed containers.

Ibuprofen Piconol

イブプロフェンピコノール



$C_{19}H_{23}NO_2$: 297.39
Pyridin-2-ylmethyl (2*RS*)-2-[4-(2-methylpropyl)phenyl]propanoate
[*64622-45-3*]

Ibuprofen Piconol contains not less than 98.5% and not more than 101.0% of ibuprofen piconol ($C_{19}H_{23}NO_2$), calculated on the anhydrous basis.

Description Ibuprofen Piconol occurs as a clear, colorless to pale yellowish liquid. It is odorless or has a slight characteristic odor.

It is miscible with methanol, with ethanol (95), with acetone and with acetic acid (100).

It is practically insoluble in water.

It decomposes on exposure to light.

It shows no optical rotation.

Identification **(1)** Dissolve 10 mg of Ibuprofen Piconol in 250 mL of ethanol (95). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ibuprofen Piconol as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.529 – 1.532

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.046 – 1.050

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 0.5 g of Ibuprofen Piconol in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate ⟨*1.14*⟩—Dissolve 0.5 g of Ibuprofen Piconol in 20 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 20 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.038%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 4.0 g of Ibuprofen Piconol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(4) Related substances—Dissolve 0.10 g of Ibuprofen Piconol in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate, acetic acid (100) and methanol (30:10:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 10) on the plate, and heat at 170°C for 10 minutes: the number of spots other than the dark brown principal spot obtained from the sample solution is two or less, and they are not more intense than the dark brown spot obtained from the standard solution.

Water ⟨*2.48*⟩ Not more than 0.1% (5 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Ibuprofen Piconol, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.74 mg of $C_{19}H_{23}NO_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ibuprofen Piconol Cream

イブプロフェンピコノールクリーム

Ibuprofen Piconol Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of ibuprofen piconol ($C_{19}H_{23}NO_2$: 297.39).

Method of preparation Prepare as directed under Creams, with Ibuprofen Piconol.

Identification To an amount of Ibuprofen Piconol Cream, equivalent to 50 mg of Ibuprofen Piconol, add 10 mL of methanol, warm in a water bath, mix well, filter after cooling, and use the filtrate as the sample solution. Separately, dissolve 50 mg of ibuprofen piconol in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (15:5:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

pH Being specified separately when the drug is granted ap-

proval based on the Law.

Assay Weigh accurately an amount of Ibuprofen Piconol Cream, equivalent to about 15 mg of ibuprofen piconol ($C_{19}H_{23}NO_2$), add 10 mL of tetrahydrofuran for liquid chromatography, shake vigorously, and add exactly 10 mL of the internal standard solution. Then, add methanol to make 30 mL, shake vigorously, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.15 g of ibuprofen piconol for assay (separately determine the water <2.48> in the same manner as Ibuprofen Piconol), and dissolve in tetrahydrofuran for liquid chromatography to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 30 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ibuprofen piconol to that of the internal standard.

$$\text{Amount (mg) of ibuprofen piconol } (C_{19}H_{23}NO_2) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of ibuprofen piconol for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of triphenylmethane in methanol (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and acetic acid-sodium acetate buffer solution (pH 4.0) (3:1).

Flow rate: Adjust so that the retention time of ibuprofen piconol is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ibuprofen piconol and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ibuprofen piconol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ibuprofen Piconol Ointment

イブプロフェンピコノール軟膏

Ibuprofen Piconol Ointment contains not less than 95.0% and not more than 105.0% of the labeled amount of ibuprofen piconol ($C_{19}H_{23}NO_2$: 297.39).

Method of preparation Prepare as directed under Ointments, with Ibuprofen Piconol.

Identification To an amount of Ibuprofen Piconol Ointment, equivalent to 50 mg of Ibuprofen Piconol, add 10 mL of methanol, warm at 60°C in a water bath, mix well, and filter after cooling. Use the filtrate as the sample solution. Separately, dissolve 50 mg of ibuprofen piconol in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (15:5:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

Assay Weigh accurately an amount of Ibuprofen Piconol Ointment, equivalent to about 15 mg of ibuprofen piconol ($C_{19}H_{23}NO_2$), add 10 mL of tetrahydrofuran for liquid chromatography, shake vigorously, and add exactly 10 mL of the internal standard solution. Then, add methanol to make 30 mL, shake vigorously, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.15 g of ibuprofen piconol for assay (separately determine the water <2.48> in the same manner as Ibuprofen Piconol), and dissolve in tetrahydrofuran for liquid chromatography to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make exactly 30 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ibuprofen piconol to that of the internal standard.

$$\text{Amount (mg) of ibuprofen piconol } (C_{19}H_{23}NO_2) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of ibuprofen piconol for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of triphenylmethane in methanol (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and acetic acid-sodium acetate buffer solution (pH 4.0) (3:1).

Flow rate: Adjust so that the retention time of ibuprofen piconol is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ibuprofen piconol and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ibuprofen piconol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ichthammol

イクタモール

Ichthammol, calculated on the dried basis, contains not less than 2.5% of ammonia (NH_3: 17.03), not more than 8.0% of ammonium sulfate [$(NH_4)_2SO_4$: 132.14], and not less than 10.0% of total sulfur (as S: 32.07).

Description Ichthammol is a red-brown to blackish brown, viscous fluid. It has a characteristic odor.

It is miscible with water.

It is partially soluble in ethanol (95) and in diethyl ether.

Identification **(1)** To 4 mL of a solution of Ichthammol (3 in 10) add 8 mL of hydrochloric acid: a yellow-brown to blackish brown, oily or resinous mass is produced. Cool the mass with ice to solidify, and discard the water layer. Wash the residue with diethyl ether: a part of the mass dissolves but it does not dissolve completely even when it is washed until almost no color develops in the washing. Perform the following tests with this residue.

(i) To 0.1 g of the residue add 1 mL of a mixture of ethanol (95) and diethyl ether (1:1): it dissolves.

(ii) To 0.1 g of the residue add 2 mL of water: it dissolves. To 1 mL of this solution add 0.4 mL of hydrochloric acid: a yellow-brown to blackish brown oily or resinous substance is produced.

(iii) To 1 mL of the solution obtained in (ii) add 0.3 g of sodium chloride: a yellow-brown or blackish brown oily or resinous substance is produced.

(2) Boil 2 mL of a solution of Ichthammol (1 in 10) with 2 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

Loss on drying ⟨*2.41*⟩ Not more than 50% (0.5 g, 105°C, 6 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

Assay **(1)** Ammonia—Weigh accurately about 5 g of Ichthammol, transfer to a Kjeldahl flask, and add 60 mL of water, 1 mL of 1-octanol and 4.5 mL of a solution of sodium hydroxide (2 in 5). Connect the flask to a distilling tube with a spray trap and a condenser, and immerse the lower outlet of the condenser in the receiver containing exactly 30 mL of 0.25 mol/L sulfuric acid VS. Distil slowly, collect about 50 mL of the distillate, and titrate ⟨*2.50*⟩ the excess sulfuric acid with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.25 mol/L sulfuric acid VS
= 8.515 mg of NH_3

(2) Ammonium sulfate—Weigh accurately about 1 g of Ichthammol, add 25 mL of ethanol (95), stir thoroughly, and filter. Wash with a mixture of ethanol (95) and diethyl ether (1:1) until the washings are clear and colorless. Dry the filter paper and the residue in air, dissolve the residue in 200 mL of hot water acidified slightly with hydrochloric acid, and filter. Boil the filtrate, add 30 mL of barium chloride TS slowly, heat for 30 minutes on a water bath, and filter. Wash the precipitate with water, dry, and ignite to constant mass. Weigh the residue as barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of ammonium sulfate $[(NH_4)_2SO_4]$
= amount (mg) of barium sulfate ($BaSO_4$) × 0.566

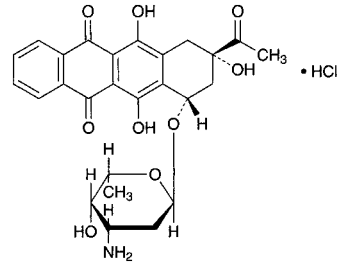
(3) Total sulfur—Weigh accurately about 0.6 g of Ichthammol, transfer to a 200-mL Kjeldahl flask, and add 30 mL of water and 5 g of potassium chlorate, then add slowly 30 mL of nitric acid, and evaporate the mixture to about 5 mL. Transfer the residue to a 300-mL beaker with the aid of 25 mL of hydrochloric acid, and evaporate again to 5 mL. Add 100 mL of water, boil, filter, and wash with water. Heat the combined filtrate and washings to boil, add gradually 30 mL of barium chloride TS, heat the mixture on a water bath for 30 minutes, and filter. Wash the precipitate with water, dry, and ignite to constant mass. Weigh the residue as barium sulfate ($BaSO_4$).

Amount (mg) of total sulfur (S)
= amount (mg) of barium sulfate ($BaSO_4$) × 0.13739

Containers and storage Containers—Tight containers.

Idarubicin Hydrochloride

イダルビシン塩酸塩



$C_{26}H_{27}NO_9$.HCl: 533.95
(2*S*,4*S*)-2-Acetyl-4-(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyloxy)-2,5,12-trihydroxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride
[*57852-57-0*]

Idarubicin Hydrochloride contains not less than 960 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the anhydrous basis. The potency of Idarubicin Hydrochloride is expressed as mass (potency) of idarubicin hydrochloride ($C_{26}H_{27}NO_9$.HCl).

Description Idarubicin Hydrochloride occurs as a yellow-red powder.

It is sparingly soluble in methanol, slightly soluble in water and in ethanol (95), and practically insoluble in acetonitrile and in diethyl ether.

Identification **(1)** Determine the absorption spectra of a solution of Idarubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Idarubicin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Idarubicin Hydrochloride and Idarubicin Hydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 2 mg of Idarubicin Hydrochloride in 3 mL

of water, and add 1 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white turbidity is produced.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +188 – +201° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 10 mg of Idarubicin Hydrochloride in 10 mL of water: the pH of the solution is between 5.0 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 10 mg of Idarubicin Hydrochloride in 10 mL of water: the solution is clear and yellowish red in color.

(2) Silver— Dissolve exactly 0.10 g of Idarubicin Hydrochloride in diluted nitric acid (1 in 200) to make exactly 20 mL, and use this solution as the sample solution. Separately, to exactly 5 mL of Standard Silver Solution for Atomic Absorption Spectrophotometry add diluted nitric acid (1 in 200) to make exactly 50 mL. Pipet a suitable amount of this solution, dilute exactly it with diluted nitric acid (1 in 200) so that each mL contains 0.05 μg, 0.075 μg, 0.1 μg and 0.2 μg of silver (Ag: 107.87), and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions, and calculate the amount of silver in the sample solution using the calibration curve obtained with the absorbances of the standard solution: not more than 20 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Silver hollow-cathode lamp.
Wavelength: 328.1 nm.

(3) Related substances—Conduct this procedure using light-resistant vessels. Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak other than idarubicin is not more than 1.0%, and the total amount of the peaks other than idarubicin is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.3 times as long as the retention time of idarubicin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase without sodium lauryl sulfate to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mobile phase without sodium lauryl sulfate to make exactly 20 mL. Confirm that the peak area of idarubicin obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of idarubicin are not less than 3000 and 0.8 to 1.2, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of idarubicin is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 5.0% (0.1 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (2 g).

Assay Weigh accurately an amount of Idarubicin Hydrochloride and Idarubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in the mobile phase prepared without addition of sodium lauryl sulfate to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of idarubicin in each solution.

Amount [μg (potency)] of idarubicin hydrochloride ($C_{26}H_{27}NO_9$.HCl)
$= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Idarubicin Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 10.2 g of potassium dihydrogenphosphate in a suitable amount of water, add 1 mL of phosphoric acid and water to make 750 mL, and add 250 mL of tetrahydrofuran. To 500 mL of this solution add 0.72 g of sodium lauryl sulfate and 0.5 mL of *N*,*N*-dimethyl-*n*-octylamine, and adjust to pH 4 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust so that the retention time of idarubicin is about 15 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of idarubicin is not less than 3000.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of idarubicin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Idarubicin Hydrochloride for Injection

注射用イダルビシン塩酸塩

Idarubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of idarubicin hydrochloride ($C_{26}H_{27}NO_9$.HCl: 533.95).

Method of preparation Prepare as directed under Injections, with Idarubicin Hydrochloride.

Description Idarubicin Hydrochloride for Injection occurs as yellow-red masses.

Identification **(1)** Dissolve an amount of Idarubicin Hy-

drochloride for Injection, equivalent to 2 mg (potency) of Idarubicin Hydrochloride, in 5 mL of sodium hydroxide TS: the solution shows a blue-purple color.

(2) Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 1 mg (potency) of Idarubicin Hydrochloride, in 1 mL of water, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 250 nm and 254 nm, between 285 nm and 289 nm, between 480 nm and 484 nm, and between 510 nm and 520 nm.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of Idarubicin Hydrochloride, in 5 mL of water is between 5.0 and 7.0.

Purity Clarity and color of solution—Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of Idarubicin Hydrochloride, in 5 mL of water: the solution is clear and yellow-red.

Water <*2.48*> Weigh accurately the mass of 1 Idarubicin Hydrochloride for Injection, add 5 mL of methanol for water determination using a syringe, dissolve with thorough shaking, and perform the test with 4 mL of this solution as directed in the Volumetric titration (direct titration). Use 4 mL of methanol for water determination as the blank. Determine the mass of the content from the difference between the mass of 1 Idarubicin Hydrochloride for Injection obtained above and the mass of its bottle and rubber stopper, which are weighed accurately after washing with water then with ethanol (95), drying at 105°C for 1 hour and allowing to cool to room temperature in a desiccator (not more than 4.0%).

Bacterial endotoxins <*4.01*> Less than 8.9 EU/mg (potency).

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 Idarubicin Hydrochloride for Injection add the mobile phase prepared without addition of sodium lauryl sulfate to make exactly V mL so that each mL contains 0.2 mg (potency) of idarubicin hydrochloride ($C_{26}H_{27}NO_9$.HCl), and use this solution as the sample solution. Separately, weigh accurately an amount of Idarubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve in the mobile phase without sodium lauryl sulfate to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Idarubicin Hydrochloride.

Amount [mg (potency)] of idarubicin hydrochloride ($C_{26}H_{27}NO_9$.HCl)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount [mg (potency)] of Idarubicin Hydrochloride RS taken

Foreign insoluble matter <*6.06*> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Idarubicin Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 5 mg (potency), dissolve in the mobile phase prepared without addition of sodium lauryl sulfate to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Idarubicin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve in the mobile phase without sodium lauryl sulfate to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Idarubicin Hydrochloride.

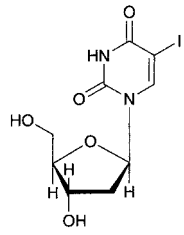
Amount [mg (potency)] of idarubicin hydrochloride ($C_{26}H_{27}NO_9$.HCl)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount [mg (potency)] of Idarubicin Hydrochloride RS taken

Containers and storage Containers—Hermetic containers.

Idoxuridine

イドクスウリジン



$C_9H_{11}IN_2O_5$: 354.10
5-Iodo-2′-deoxyuridine
[*54-42-2*]

Idoxuridine, when dried, contains not less than 98.0% of idoxuridine ($C_9H_{11}IN_2O_5$).

Description Idoxuridine occurs as colorless, crystals or a white, crystalline powder. It is odorless.

It is freely soluble in dimethylamide, slightly soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: about 176°C (with decomposition).

Identification **(1)** Dissolve 0.01 g of Idoxuridine in 5 mL of water by warming, add 5 mL of diphenylamine-acetic acid TS, and heat for 5 minutes: a blue color develops.

(2) Heat 0.1 g of Idoxuridine: a purple gas evolves.

(3) Dissolve 2 mg of Idoxuridine in 50 mL of 0.01 mol/L sodium hydroxide. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Idoxuridine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +28 – +31° (after drying, 0.2 g, sodium hydroxide TS, 20 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Idoxuridine in 5 mL of a solution of sodium hydroxide (1 in 200): the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Idoxuridine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Idoxuridine

in exactly 10 mL of a mixture of dilute ethanol and ammonia solution (28) (99:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 50 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diluted 2-propanol (2 in 3) (4:1) to a distance of about 10 cm, and air-dry the plate. Then develop two-dimensionally at right angles to the first, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

(4) Iodine and iodide—Dissolve 0.10 g of Idoxuridine in 20 mL of water and 5 mL of sodium hydroxide TS, and add immediately 5 mL of dilute sulfuric acid under ice-cooling. Allow to stand for 10 minutes with occasional shaking, and filter. Transfer the filtrate into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100), shake for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh accurately 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. To exactly 1 mL of this solution add 19 mL of water, 5 mL of sodium hydroxide TS and 5 mL of dilute sulfuric acid, mix, and filter. Transfer the filtrate to a Nessler tube, and proceed in the same manner.

Loss on drying <2.41> Not more than 0.5% (2 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.7 g of Idoxuridine, previously dried, dissolve in 80 mL of *N*,*N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 35.41 mg of $C_9H_{11}IN_2O_5$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Idoxuridine Ophthalmic Solution

イドクスウリジン点眼液

Idoxuridine Ophthalmic Solution contains not less than 90.0% and not more than 110.0% of the labeled amount of idoxuridine ($C_9H_{11}IN_2O_5$: 354.10).

Method of preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Idoxuridine.

Description Idoxuridine Ophthalmic Solution is a clear, colorless liquid.

Identification **(1)** To a volume of Idoxuridine Opthalmic Solution, equivalent to 5 mg of Idoxuridine, add 5 mL of diphenylamine-acetic acid TS, and heat for 20 minutes: a light blue color develops.

(2) Place a volume of Idoxuridine Ophthalmic Solution, equivalent to 5 mg of Idoxuridine, in a porcelain crucible, add 0.1 g of anhydrous sodium carbonate, heat slowly, evaporate to dryness and ignite until the residue is incinerated. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and add 2 to 3 drops of sodium nitrite TS: a yellow-brown color develops. Then add 2 to 3 drops of starch TS: a deep blue color develops.

(3) To a volume of Idoxuridine Ophthalmic Solution, equivalent to 2 mg of Idoxuridine, add 0.01 mol/L sodium hydroxide TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 281 nm.

pH <2.54> 4.5 – 7.0

Purity 5-Iodouracil and 2′-deoxyuridine—To a volume of Idoxuridine Ophthalmic Solution, equivalent to 4.0 mg of Idoxuridine, add water to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve 12.0 mg of 5-iodouracil for liquid chromatography and 4.0 mg of 2′-deoxyuridine for liquid chromatography in water to make exactly 200 mL. Measure exactly 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of 5-iodouracil and 2′-deoxyuridine: the peak areas of 5-iodouracil and 2′-deoxyuridine of the sample solution are not larger than the peak areas of 5-iodouracil and 2′-deoxyuridine of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (24:1).

Flow rate: Adjust so that the retention time of 2′-deoxyuridine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 2′-deoxyuridine and 5-iodouracil are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 2′-deoxyuridine is not more than 1.0%.

Foreign insoluble matter <6.11> It meets the requirement.

Insoluble particulate matter <6.08> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Idoxuridine Ophthalmic Solution, equivalent to 3 mg of idoxuridine ($C_9H_{11}IN_2O_5$), add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Separately weigh accurately about 10 mg of Idoxuridine RS, previously dried at 60°C for 3 hours, dissolve in water to make exactly 10 mL. Measure exactly 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL

each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of idoxuridine to that of the internal standard, respectively.

$$\text{Amount (mg) of idoxuridine } (C_9H_{11}IN_2O_5) = M_S \times Q_T/Q_S \times 3/10$$

M_S: Amount (mg) of Idoxuridine RS taken

Internal standard solution—A solution of sulfathiazole in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (87:13).

Flow rate: Adjust so that the retention time of idoxuridine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, idoxuridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

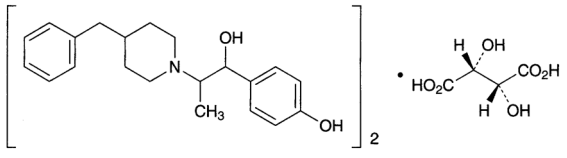
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of idoxuridine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, in a cold place, and avoid freezing.

Ifenprodil Tartrate

イフェンプロジル酒石酸塩



$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$: 800.98

(1*RS*,2*SR*)-4-[2-(4-Benzylpiperidin-1-yl)-1-hydroxypropyl]phenol hemi-(2*R*,3*R*)-tartrate [*23210-58-4*]

Ifenprodil Tartrate contains not less than 98.5% of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$, calculated on the anhydrous basis.

Description Ifenprodil Tartrate occurs as a white crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in ethanol (95), slightly soluble in water and in methanol, and practically insoluble in diethyl ether.

Optical rotation $[\alpha]_D^{20}$: +11 – +15° (1 g calculated on the anhydrous basis, ethanol (95), 20 mL, 100 mm).

Melting point: about 148°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ifenprodil Tartrate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ifenprodil Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.4 g of Ifenprodil Tartrate in 40 mL of water by warming. After cooling, add 0.5 mL of ammonia TS to this solution, extract with two 40-mL portions of chloroform, and collect the water layer. Evaporate 30 mL of the water layer on a water bath to dryness, and after cooling, dissolve the residue in 6 mL of water: the solution responds to the Qualitative Tests <*1.09*> for tartrate.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Ifenprodil Tartrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.30 g of Ifenprodil Tartrate in 10 mL of diluted ethanol (95) (3 in 4), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (3 in 4) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thinlayer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, 1-butanol and ammonia solution (28) (140:40:20:1) to a distance of about 10 cm, and air-dry the plate. Spray hydrogen hexachloroplatinate (IV)-potassium iodide TS evenly on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ifenprodil Tartrate, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 40.05 \text{ mg of } (C_{21}H_{27}NO_2)_2.C_4H_6O_6$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Ifenprodil Tartrate Fine Granules

イフェンプロジル酒石酸塩細粒

Ifenprodil Tartrate Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6$: 800.98].

Method of preparation Prepare as directed under Granules, with Ifenprodil Tartrate.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 274 nm and 278 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: Ifenprodil Tartrate Fine Granules in single-dose packages meet the requirement of the Content uniformity test.

To the total amount of the content of 1 package of Ifenprodil Tartrate Fine Granules, add 10 mL of water and a suitable amount of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly V mL so that each mL contains about 0.1 mg of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$. Filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6] = M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of ifenprodil tartrate for assay taken, calculated on the anhydrous basis

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Powder Ifenprodil Tartrate Fine Granules, and weigh accurately a portion of the powder, equivalent to about 10 mg of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$, add 5 mL of water and a suitable amount of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly 100 mL. Filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of ifenprodil tartrate for assay (separately determine the water <2.48> in the same manner as Ifenprodil Tartrate), add 10 mL of water and a mixture of ethanol (99.5) and water (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ifenprodil in each solution.

Amount (mg) of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6] = M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of ifenprodil tartrate for assay taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 224 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 6.5 with potassium hydroxide TS, and add water to make 1000 mL. To 420 mL of this solution add 320 mL of methanol for liquid chromatography and 260 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of ifenprodil is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ifenprodil are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ifenprodil is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ifenprodil Tartrate Tablets

イフェンプロジル酒石酸塩錠

Ifenprodil Tartrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6$: 800.98].

Method of preparation Prepare as directed under Tablets, with Ifenprodil Tartrate.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 274 nm and 278 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ifenprodil Tartrate Tablets, add $V/20$ mL of water, and shake until the tablet is completely disintegrated. Then, add $7V/10$ mL of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly V mL so that each mL contains about 0.1 mg of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$. Filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6] = M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of ifenprodil tartrate for assay taken, calculated on the anhydrous basis

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately the mass of not less than 20 Ifenprodil Tartrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2C_4H_6O_6]$, add 5 mL of water and a suitable amount of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly 100 mL. Filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of ifenprodil tartrate for assay (separately determine the water <2.48> in the same manner as Ifenprodil Tartrate), add 10 mL of water and a mixture of ethanol (99.5) and water (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test

with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of ifenprodil tartrate in each solution.

Amount (mg) of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$ $= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of ifenprodil tartrate for assay taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 224 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 6.5 with potassium hydroxide TS, and add water to make 1000 mL. To 420 mL of this solution, add 320 mL of methanol for liquid chromatography and 260 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of ifenprodil is about 10 minutes.

System suitability—

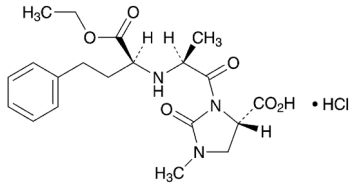
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ifenprodil are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ifenprodil is not more than 1.0%.

Containers and storage containers—Tight containers.

Imidapril Hydrochloride

イミダプリル塩酸塩



$C_{20}H_{27}N_3O_6.HCl$: 441.91

(4*S*)-3-{(2*S*)-2-[(1*S*)-1-Ethoxycarbonyl-3-phenylpropylamino]propanoyl}-1-methyl-2-oxoimidazolidine-4-carboxylic acid monohydrochloride

[*89396-94-1*]

Imidapril Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of imidapril hydrochloride ($C_{20}H_{27}N_3O_6.HCl$).

Description Imidapril Hydrochloride occurs as a white crystals.

It is freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).

Dissolve 1.0 g of Imidapril Hydrochloride in 100 mL of water: the pH of the solution is about 2.

Melting point: about 203°C (with decomposition).

Identification **(1)** To 3 mL of a solution of Imidapril Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Imidapril Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Imidapril Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −65.0 – −69.0° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Imidapril Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Imidapril Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.45 to imidapril, obtained from the sample solution, is not larger than 2/5 times the peak area of imidapril obtained from the standard solution, and the area of each peak other than imidapril and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of imidapril from the standard solution. Furthermore, the total area of the peaks other than imidapril from the sample solution is not larger than 1/2 times the peak area of imidapril from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.7 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.

Flow rate: Adjust so that the retention time of imidapril is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of imidapril, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of imidapril obtained from 20 μL of this solution is equivalent to 7 to 13% of that of imidapril obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times

with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imidapril is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Imidapril Hydrochloride, previously dried, dissolve in 70 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS from the first equivalent point to the second equivalent point (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 44.19 mg of $C_{20}H_{27}N_3O_6$.HCl

Containers and storage Containers—Well-closed containers.

Imidapril Hydrochloride Tablets

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Imidapril Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of Imidapril Hydrochloride ($C_{20}H_{27}N_3O_6$.HCl: 441.91).

Method of preparation Prepare as directed under Tablets, with Imidapril Hydrochloride.

Identification Weigh accurately an amount of powdered Imidapril Hydrochloride Tablets, equivalent to 25 mg of Imidapril Hydrochloride, add 5 mL of ethanol (99.5), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 25 mg of imidapril hydrochloride in 5 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, ethyl acetate, water, ethanol (99.5) and acetic acid (100) (16:16:7:2:2) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same *R*f value as the spot obtained from the standard solution.

Purity Related substances—To a quantity of powdered Imidapril Hydrochloride Tablets, equivalent to 25 mg of Imidapril Hydrochloride, add 40 mL of diluted methanol (2 in 5), shake vigorously for 10 minutes, add diluted ethanol (2 in 5) to make 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (2 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.45 to imidapril, obtained from the sample solution, is not larger than the peak area of imidapril obtained from the standard solution, the area of the peak having the relative retention time of about 0.8 to imidapril from the sample solution is not larger than 7/10 times the peak area of imidapril from the standard solution, and the area of each peak other than imidapril and the peaks mentioned above from the sample solution is not larger than 3/10 times the peak area of imidapril from the standard solution. Furthermore, the total area of the peaks other than imidapril from the sample solution is not larger than 1.5 times the peak area of imidapril from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of imidapril, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add diluted methanol (2 in 5) to make exactly 20 mL. Confirm that the peak area of imidapril obtained from 20 μL of this solution is equivalent to 7 to 13% of that of imidapril obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imidapril is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Imidapril Hydrochloride Tablets add $2V/5$ mL of water, shake vigorously for 10 minutes, add diluted methanol (2 in 3) to make exactly V mL so that each mL contains about 0.1 mg of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$.HCl), filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of imidapril for assay, previously dried at 105°C for 3 hours, dissolve in diluted methanol (2 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of imidapril in each solution.

Amount (mg) of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$.HCl)
$= M_S \times A_T/A_S \times V/100$

M_S: Amount (mg) of imidapril hydrochloride for assay taken

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imidapril is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Imidapril Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Imidapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of imidapril hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of imidapril in each solution.

Dissolution rate (%) with respect to the labeled amount of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of imidapril hydrochloride for assay taken

C: Labeled amount (mg) of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$.HCl) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imidapril is not more than 2.0%.

Assay Weigh accurately not less than 20 Imidapril Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$.HCl), add 30 mL of diluted methanol (2 in 5) and exactly 5 mL of the internal standard solution, shake vigorously for 10 minutes, add diluted methanol (2 in 5) to make 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add diluted methanol (2 in 5) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of imidapril hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in exactly 5 mL of the internal standard solution, add diluted methanol (2 in 5) to make 50 mL. Pipet 5 mL of this solution, add diluted methanol (2 in 5) to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of imidapril to that of the internal standard.

Amount (mg) of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$.HCl)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of imidapril hydrochloride for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (2 in 5) (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.7 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.

Flow rate: Adjust so that the retention time of imidapril is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, imidapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of imidapril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Imipenem Hydrate

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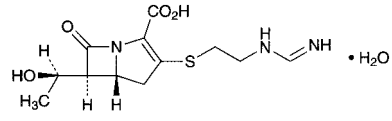

$C_{12}H_{17}N_3O_4S.H_2O$: 317.36

(5*R*,6*S*)-3-[2-(Formimidoylamino)ethylsulfanyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate

[*74431-23-5*]

Imipenem Hydrate contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Imipenem Hydrate is expressed as mass (potency) of imipenem ($C_{12}H_{17}N_3O_4S$: 299.35).

Description Imipenem Hydrate occurs as white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Imipenem Hydrate in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Imipenem RS

prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Imipenem Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Imipenem RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +89 – +94° (50 mg calculated on the anhydrous basis, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0), 10 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Imipenem Hydrate in 200 mL of water is between 4.5 and 7.0.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Imipenem Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Put 2.0 g of Imipenem Hydrate in a crucible, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes evolve. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure once more. Then add 2 mL of hydrogen peroxide (30), heat, and repeat this procedure several times until the color of the solution changes to colorless to pale yellow. After cooling, heat again until white fumes evolve. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 1 ppm).

(3) Related substances—Dissolve 50 mg of Imipenem Hydrate in 50 mL of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of thienamycin, having the relative retention time of about 0.8 to imipenem, obtained from the sample solution is not larger than 1.4 times the peak area of imipenem obtained from the standard solution, the area of the peak other than imipenem and thienamycin from the sample solution is not larger than 1/3 times the peak area of imipenem from the standard solution, and the total area of the peaks other than imipenem and thienamycin from the sample solution is not larger than the peak area of imipenem from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of imipenem.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 5 mL of the standard solution, add 0.1 mol/L 3-(*N*-morpholino)-propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL. Confirm that the peak area of imipenem obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 2.0%.

Water ⟨*2.48*⟩ Not less than 5.0% and not more than 8.0% (20 mg, coulometric titration, water evaporation temperature: 140°C).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Perform the procedure within 30 minutes after preparation of the sample solution and standard solution. Weigh accurately an amount of Imipenem Hydrate and Imipenem RS, equivalent to about 50 mg (potency), dissolve each in 0.1 mol/L 3-(*N*-morpholino)-propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution, within 30 minutes after preparation of these solutions, as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of imipenem in each solution.

$$\text{Amount [}\mu\text{g (potency)] of imipenem } (C_{12}H_{17}N_3O_4S) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Imipenem RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) and acetonitrile (100:1).

Flow rate: Adjust so that the retention time of imipenem is about 6 minutes.

System suitability—

System performance: Dissolve 50 mg of Imipenem Hydrate and 75 mg of resorcinol in 50 mL of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0). When the procedure is run with 10 μL of this solution under the above operating conditions, imipenem and resorcinol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 0.80%.

Containers and storage Containers—Hermetic containers.

Imipenem and Cilastatin Sodium for Injection

注射用イミペネム·シラスタチンナトリウム

Imipenem and Cilastatin Sodium for Injection is a preparation for injection which is dissolved or suspended before use.

It contains not less than 93.0% and not more than 115.0% of the labeled potency of imipenem ($C_{12}H_{17}N_3O_4S$: 299.35) and an amount of cilastatin sodium ($C_{16}H_{25}N_2NaO_5S$: 380.43), equivalent to not less than 93.0% and not more than 115.0% of the labeled amount of cilastatin ($C_{16}H_{26}N_2O_5S$: 358.45).

Method of preparation Prepare as directed under Injections, with Imipenem Hydrate and Cilastatin Sodium.

Description Imipenem and Cilastatin Sodium for Injection occurs as a white to light yellowish white powder.

Identification (1) To 1 mL of a solution of Imipenem and Cilastatin Sodium for Injection (1 in 100) add 1 mL of ninhydrin TS, heat in a water bath for 5 minutes: a purple color appears (cilastatin).

(2) To 2 mL of a solution of Imipenem and Cilastatin Sodium for Injection (1 in 1000) add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 296 nm and 300 nm (imipenem).

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Imipenem and Cilastatin Sodium for Injection, equivalent to 0.5 g (potency) of Imipenem Hydrate, in 100 mL of isotonic sodium chloride solution is between 6.5 and 8.0. The pH of the Injection intended for intramuscular use is between 6.0 and 7.5.

Purity Clarity and color of solution—Dissolve an amount of Imipenem and Cilastatin Sodium for Injection, equivalent to 0.5 g (potency) of Imipenem Hydrate, in 100 mL of isotonic sodium chloride solution: the solution is clear and colorless or pale yellow.

Loss on drying ⟨*2.41*⟩ Not more than 3.0% (1 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.25 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test (*T*: 104.0%).

Dissolve the total amount of the content of 1 Imipenem and Cilastatin Sodium for Injection in isotonic sodium chloride solution to make exactly 100 mL. Measure exactly *V* mL of this solution, equivalent to about 25 mg (potency) of Imipenem Hydrate, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the sample solution. Proceed hereafter as directed in the Assay.

Amount [mg (potency)] of imipenem ($C_{12}H_{17}N_3O_4S$)
$= M_{SI} \times A_{TI}/A_{SI} \times 100/V$

Amount (mg) of cilastatin ($C_{16}H_{26}N_2O_5S$)
$= M_{SC} \times A_{TC}/A_{SC} \times 100/V \times 0.955$

M_{SI}: Amount [mg (potency)] of Imipenem RS taken
M_{SC}: Amount (mg) of cilastatin ammonium for assay taken, calculated on anhydrous and ethanol-free basis

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: It meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ Perform the test according to the Method 1: the Injection which is dissolved before use meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Imipenem and Cilastatin Sodium for Injections. Weigh accurately an amount of the content, equivalent to 1 Imipenem and Cilastatin Sodium for Injection, dissolve in isotonic sodium chloride solution to make exactly 100 mL. Measure exactly an amount of this solution, equivalent to about 25 mg (potency) of imipenem, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL, and use this as the sample solution. Separately, weigh accurately an amount of Imipenem RS, equivalent to about 25 mg (potency), and weigh accurately about 25 mg of cilastatin ammonium for assay, dissolve in 10 mL of isotonic sodium chloride solution, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_{TI} and A_{SI} of imipenem, and those, A_{TC} and A_{SC} of cilastatin in each solution.

Amount [mg (potency)] of imipenem ($C_{12}H_{17}N_3O_4S$)
$= M_{SI} \times A_{TI}/A_{SI}$

Amount (mg) of cilastatin ($C_{16}H_{26}N_2O_5S$)
$= M_{SC} \times A_{TC}/A_{SC} \times 0.955$

M_{SI}: Amount [mg (potency)]of Imipenem RS taken
M_{SC}: Amount (mg) of cilastatin ammonium for assay taken, calculated on anhydrous and ethanol-free basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 0.836 g of 3-(*N*-morpholino)propanesulfonic acid, 1.0 g of sodium 1-hexane sulfonate and 50 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 800 mL of water, adjust to pH 7.0 with 0.1 mol/L sodium hydrate TS, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of imipenem is about 3 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, imipenem and cilastatin are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factors of the peak of imipenem and cilastatin are not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times

with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of imipenem and cilastatin are not more than 2.0%, respectively.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Imipramine Hydrochloride

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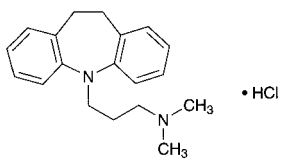


$C_{19}H_{24}N_2.HCl$: 316.87
3-(10,11-Dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropylamine monohydrochloride
[*113-52-0*]

Imipramine Hydrochloride, when dried, contains not less than 98.5% of imipramine hydrochloride ($C_{19}H_{24}N_2.HCl$).

Description Imipramine Hydrochloride occurs as a white to pale yellowish white crystalline powder. It is odorless.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of 0.1 g of Imipramine Hydrochloride in 10 mL of water is between 4.2 and 5.2.

It is gradually colored by light.

Identification **(1)** Dissolve 5 mg of Imipramine Hydrochloride in 2 mL of nitric acid: a deep blue color develops.

(2) Dissolve 5 mg of Imipramine Hydrochloride in 250 mL of 0.01 mol/L hydrochloric acid TS. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Imipramine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dissolve 0.05 g of Imipramine Hydrochloride in 5 mL of water, add 1 mL of ammonia TS, allow to stand for 5 minutes, filter, and acidify the filtrate with dilute nitric acid: it responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Melting point ⟨*2.60*⟩ 170 – 174°C (with decomposition).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Imipramine Hydrochloride in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: Take exactly 1.0 mL of Cobalt (II) Chloride CS, 2.4 mL of Iron (III) Chloride CS, 0.4 mL of Copper (II) Sulfate CS and 6.2 mL of diluted hydrochloric acid (1 in 40), and mix them. Pipet 0.5 mL of this solution, and add exactly 9.5 mL of water.

(2) Iminodibenzyl—Dissolve 50 mg of Imipramine Hydrochloride in 10 mL of a mixture of hydrochloric acid and ethanol (95) (1:1) in a 25-mL brown volumetric flask. Cool the flask in ice water, add 5 mL of an ethanol (95) solution of furfural (1 in 250) and 5 mL of hydrochloric acid, and allow to stand at 25°C for 3 hours. Add a mixture of hydrochloric acid and ethanol (95) (1:1) to make 25 mL, and determine the absorbance of this solution at 565 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it is not more than 0.16.

(3) Related substances—Dissolve 0.20 g of Imipramine Hydrochloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100), hydrochloric acid and water (11:7:1:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Imipramine Hydrochloride, previously dried, and dissolve in 20 mL of water. Add 5 mL of sodium hydroxide TS, and extract with three 20-mL portions of chloroform. Filter each extract through a pledget of absorbent cotton on which a small quantity of anhydrous sodium sulfate is placed. Combine the chloroform extracts, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the yellow solution changes to red-purple (indicator: 10 drops of metanil yellow TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 31.69 mg of $C_{19}H_{24}N_2.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Imipramine Hydrochloride Tablets

イミプラミン塩酸塩錠

Imipramine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2.HCl$: 316.87).

Method of preparation Prepare as directed under Tablets, with Imipramine Hydrochloride.

Identification **(1)** Weigh a quantity of powdered Imipramine Hydrochloride Tablets, equivalent to 0.25 g of Imipramine Hydrochloride, add 25 mL of chloroform, shake thoroughly, and filter. Evaporate the filtrate on a water bath, and proceed with the residue as directed in the Identification (1) under Imipramine Hydrochloride.

(2) Dissolve an amount of the residue obtained in (1), equivalent to 5 mg of Imipramine Hydrochloride, in 250 mL of 0.01 mol/L hydrochloric acid TS, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 249 nm and 253 nm, and a shoulder between 270 nm and 280 nm.

(3) Dry the residue obtained in (1) at 105°C for 2 hours: the residue melts ⟨*2.60*⟩ between 170°C and 174°C (with de-

composition).

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Imipramine Hydrochloride Tablets add exactly 40 mL of 0.01 mol/L hydrochloric acid TS, disperse the tablet into a small particles using ultrasonic waves, then shake well. Centrifuge the solution, pipet V mL of the supernatant liquid, add water to make exactly V' mL so that each mL contains about 20 μg of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Imipramine Hydrochloride RS, previously dried at 105°C for 2 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances at 251 nm, A_{T1} and A_{S1}, and at 330 nm, A_{T2} and A_{S2}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl)
$= M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times V'/V \times 4/125$

M_S: Amount (mg) of Imipramine Hydrochloride RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Imipramine Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Imipramine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL of the filtrate contains about 10 μg of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Imipramine Hydrochloride RS, previously dried at 105°C for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Imipramine Hydrochloride RS taken
C: Labeled amount (mg) of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl) in 1 tablet

Assay Take 20 Imipramine Hydrochloride Tablets, add exactly 200 mL of 0.01 mol/L hydrochloric acid TS, and shake well until the tablets are completely disintegrated. After centrifuging the solution, pipet a volume of the supernatant liquid, equivalent to about 25 mg of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl), add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Imipramine Hydrochloride RS, previously dried at 105°C for 2 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 3 mL each of these solutions into separators which contain 15 mL of potassium hydrogen phthalate buffer solution (pH 5.6), 8 mL of bromocresol green-sodium hydroxide TS and 30 mL of chloroform, and shake. Filter the chloroform layer through a pledget of absorbent cotton into a 100-mL volumetric flask. Repeat the extraction with two 30-mL portions of chloroform, combine the chloroform layers in the 100-mL volumetric flask, and add chloroform to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution obtained by proceeding with 3 mL of 0.01 mol/L hydrochloric acid TS in the same manner as the blank. Determine the absorbances, A_T and A_S, of these solutions at 416 nm.

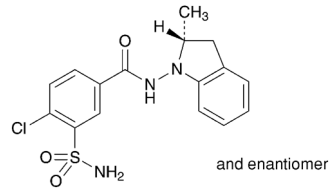
Amount (mg) of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Imipramine Hydrochloride RS taken

Containers and storage Containers—Tight containers.

Indapamide

インダパミド



$C_{16}H_{16}ClN_3O_3S$: 365.83
4-Chloro-*N*-[(2*RS*)-2-methyl-2,3-dihydro-1*H*-indol-1-yl]-3-sulfamoylbenzamide
[*26807-65-8*]

Indapamide contains not less than 98.5% and not more than 101.5% of indapamide ($C_{16}H_{16}ClN_3O_3S$), calculated on the dried basis.

Description Indapamide occurs as a white crystalline powder.

It is freely soluble in ethanol (99.5), and practically insoluble in water.

A solution of Indapamide in ethanol (99.5) (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Indapamide in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Indapamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Indapamide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Indapamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Indapamide as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Melting point ⟨*2.60*⟩ 167 – 171°C

Purity **(1)** Chloride ⟨*1.03*⟩—To 1.5 g of Indapamide add 50 mL of water, shake for 15 minutes, allow to stand in an ice bath for 30 minutes, and filter. To 30 mL of the filtrate

add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.01%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Indapamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Indapamide in 5 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add ethanol (99.5) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of ethyl acetate, cyclohexane and acetic acid (100) (100:80:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot obtained from the standard solution (1), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1) and (2), is not more than 2.0%.

Loss on drying <*2.41*> Not more than 3.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 110°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Indapamide and Indapamide RS (separately, determine the loss on drying <*2.41*> under the same condition as Indapamide), and dissolve each in a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 10 mL each of these solutions, add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of indapamide to that of the internal standard.

$$\text{Amount (mg) of indapamide } (C_{16}H_{16}ClN_3O_3S) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Indapamide RS taken, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in a mixture of water and ethanol (99.5) (1:1) (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 287 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000), acetonitrile and methanol (6:3:1).

Flow rate: Adjust so that the retention time of indapamide is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, indapamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of indapamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Indapamide Tablets

インダパミド錠

Indapamide Tablets contain not less than 93.0% and not more than 103.0% of the labeled amount of indapamide ($C_{16}H_{16}ClN_3O_3S$: 365.83).

Method of preparation Prepare as directed under Tablets, with Indapamide.

Identification To an amount of powdered Indapamide Tablets, equivalent to 10 mg of Indapamide, add 5 mL of ethyl acetate, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Indapamide RS in 5 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, cyclohexane and acetic acid (100) (100:80:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot from the standard solution show a blue-purple color and the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Indapamide Tablets add exactly V/10 mL of the internal standard solution, and add a mixture of water and ethanol (99.5) (1:1) to make V mL so that each mL contains about 0.1 mg of indapamide ($C_{16}H_{16}ClN_3O_3S$), shake to disintegrate, treat with ultrasonic waves for 10 minutes, shake again for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of indapamide } (C_{16}H_{16}ClN_3O_3S) = M_S \times Q_T/Q_S \times V/200$$

M_S: Amount (mg) of Indapamide RS taken, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in a mixture of water and ethanol (99.5) (1:1) (3 in 1000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 45 minutes of 1-mg tablet and in 90 minutes of 2-mg tablet are not less than 70%, respectively.

Start the test with 1 tablet of Indapamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 1.1 μg of indapamide ($C_{16}H_{16}ClN_3O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Indapamide RS (separately, determine the loss on drying <2.41> under the same condition as Indapamide), and dissolve in ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of indapamide in each solution.

Dissolution rate (%) with respect to the labeled amount of indapamide ($C_{16}H_{16}ClN_3O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of Indapamide RS taken, calculated on the dried basis
C: Labeled amount (mg) of indapamide ($C_{16}H_{16}ClN_3O_3S$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Indapamide.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of indapamide are not less than 3500 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of indapamide is not more than 1.5%.

Assay To 20 Indapamide Tablets add 80 mL of a mixture of water and ethanol (99.5) (1:1), shake well to disintegrate, and treat with ultrasonic waves for 10 minutes. Shake the solution for 10 minutes, and add a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet a volume of indapamide ($C_{16}H_{16}ClN_3O_3S$), equivalent to about 2 mg, and add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Indapamide RS (separately, determine the loss on drying <2.41> under the same condition as Indapamide), and dissolve in a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Indapamide.

Amount (mg) of indapamide ($C_{16}H_{16}ClN_3O_3S$)
$= M_S \times Q_T/Q_S \times 1/10$

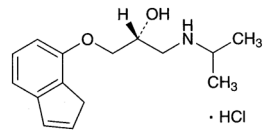
M_S: Amount [mg (potency)] of Indapamide RS taken, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in a mixture of water and ethanol (99.5) (1:1) (3 in 1000).

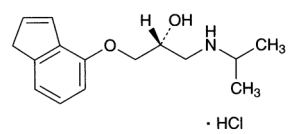
Containers and storage Containers—Tight containers.

Indenolol Hydrochloride

インデノロール塩酸塩



and enantiomer



and enantiomer

$C_{15}H_{21}NO_2.HCl$: 283.79
(2*RS*)-1-(3*H*-Inden-4-yloxy)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride
(2*RS*)-1-(3*H*-Inden-7-yloxy)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride
[*68906-88-7*]

Indenolol Hydrochloride is a mixture of (2*RS*)-1-(3*H*-Inden-4-yloxy)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride and (2*RS*)-1-(3*H*-Inden-7-yloxy)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride.

When dried, it contains not less than 98.5% of indenolol hydrochloride ($C_{15}H_{21}NO_2.HCl$).

Description Indenolol Hydrochloride occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95) and in chloroform, slightly soluble in acetic anhydride, very slightly soluble in ethyl acetate, and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Indenolol Hydrochloride in 10 mL of water is between 3.5 and 5.5.

It is colored by light.

Identification **(1)** Dissolve 0.1 g of Indenolol Hydrochloride in 1 to 2 drops of dilute hydrochloric acid and 5 mL of water, and add 1 mL of Reinecke salt TS: a red-purple precipitate is formed.

(2) Determine the absorption spectrum of a solution of Indenolol Hydrochloride (1 in 50,000) as directed under Ultravioret-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Indenolol Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Indenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Indenolol Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> $E^{1\%}_{1cm}$ (250 nm): 330 – 340 (after drying, 10 mg, water, 1000 mL).

Melting point <2.60> 140 – 143°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Indenolol Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Indenolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Indenolol Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Indenolol Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, ethanol (99.5) and ammonia solution (28) (70:15:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Isomer ratio Dissolve 5 mg of Indenolol Hydrochloride in 1.0 mL of a mixture of ethyl acetate and trifluoroacetic anhydride for gas chromatography (9:1), and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having the retention times of about 16 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: the ratio $A_a/(A_a + A_b)$ is between 0.6 and 0.7.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 2 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with 65% phenyl-methyl silicon polymer for gas chromatography at the ratio of 2%.

Column temperature: A constant temperature between 150°C and 170°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of the peak showing earlier elution of the two peaks of indenolol hydrochloride is about 16 minutes.

Selection of column: Proceed with 2 μL of the sample solution under the above operating conditions, and calculate the resolution. Use a column with the resolution between the two peaks being not less than 1.1.

Assay Weigh accurately about 0.5 g of Indenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

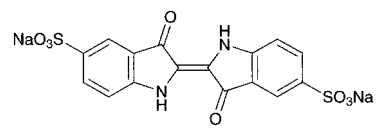
Each mL of 0.1 mol/L perchloric acid VS
= 28.38 mg of $C_{15}H_{21}NO_2.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Indigocarmine

インジゴカルミン



$C_{16}H_8N_2Na_2O_8S_2$: 466.35
Disodium 3,3′-dioxo-[$\Delta^{2,2'}$-biindoline]-5,5′-disulfonate
[*860-22-0*]

Indigocarmine, when dried, contains not less than 95.0% of indigocarmine ($C_{16}H_8N_2Na_2O_8S_2$).

Description Indigocarmine occurs as blue to dark blue, powder or granules. It is odorless.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

When compressed, it has a coppery luster.

Identification **(1)** A solution of Indigocarmine (1 in 100) is dark blue in color. Perform the following tests with this solution as the sample solution: the dark blue color of each solution disappears.

(i) Add 1 mL of nitric acid to 2 mL of the sample solution;

(ii) Add 1 mL of bromine TS to 2 mL of the sample solution;

(iii) Add 1 mL of chlorine TS to 2 mL of the sample solution;

(iv) Add 2 mL of sodium hydroxide TS and 0.2 g of zinc powder to 2 mL of the sample solution, and warm.

(2) Dissolve 0.1 g of Indigocarmine in 100 mL of a solution of ammonium acetate (1 in 650). To 1 mL of the solution add a solution of ammonium acetate (1 in 650) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Ignite 1 g of Indigocarmine to carbonize. After cooling, add 20 mL of water to the residue, shake, and filter the mixture: the filtrate responds to the Qualitative Tests <*1.09*> for sodium salt and for sulfate.

pH <*2.54*> Dissolve 0.10 g of Indigocarmine in 20 mL of water: the pH of the solution is between 5.0 and 6.0.

Purity **(1)** Water-insoluble substances—To 1.00 g of Indigocarmine add 200 mL of water, shake, and filter through a tared glass filter (G4). Wash the residue with water until the blue color of the filtrate becomes practically colorless, and dry the residue at 105°C for 4 hours: the mass of the residue does not exceed 5.0 mg.

(2) Arsenic <*1.11*>—Place 0.8 g of Indigocarmine in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and ignite gently. Repeat the addition of 2 to 3 mL of nitric acid occasionally, and continue to heat until a colorless

to light yellow solution is obtained. After cooling, add 15 mL of a saturated ammonium oxalate solution, heat the solution until dense white fumes are evolved, and concentrate to 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with 5 mL of this solution as the test solution (not more than 5 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 10.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not less than 28% and not more than 38% (after drying, 1 g).

Assay Weigh accurately about 0.5 g of Indigocarmine, previously dried, add 15 g of sodium hydrogen tartrate monohydrate, and dissolve in 200 mL of water, boil with bubbling of a stream of carbon dioxide, and titrate ⟨*2.50*⟩, while being hot, with 0.1 mol/L titanium (III) chloride VS until the color of the solution changes from blue through yellow to orange.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 23.32 mg of $C_{16}H_8N_2Na_2O_8S_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Indigocarmine Injection

インジゴカルミン注射液

Indigocarmine Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of indigocarmine ($C_{16}H_8N_2Na_2O_8S_2$: 466.35).

Method of preparation Prepare as directed under Injection, with Indigocarmine.

Description Indigocarmine Injection is a dark blue liquid.
pH: 3.0 – 5.0

Identification **(1)** To a volume of Indigocarmine Injection, equivalent to 20 mg of Indigocarmine, add 1 mL of nitric acid: the dark blue color of the liquid disappears, and a yellow-brown color develops.

(2) To a volume of Indigocarmine Injection, equivalent to 20 mg of Indigocarmine, add 1 mL of bromine TS: the dark blue color disappears, and a yellow-brown color develops.

(3) To a volume of Indigocarmine Injection, equivalent to 20 mg of Indigocarmine, add 1 mL of chlorine TS: the dark blue color disappears, and a yellow-brown color develops.

(4) To a volume of Indigocarmine Injection, equivalent to 10 mg of Indigocarmine, add ammonium acetate solution (1 in 650) to make 1000 mL, and determine the absorbance of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 610 nm and 614 nm.

Bacterial endotoxins ⟨*4.01*⟩ Less than 7.5 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ Perform the test according to Method 2: it meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Indigocarmine Injection, equivalent to about 0.2 g of indigocarmine ($C_{16}H_8N_2Na_2O_8S_2$), add 6 g of sodium hydrogen tartrate monohydrate, and dissolve in water to make 200 mL. Then boil under a carbon dioxide stream, and proceed as directed in the Assay under Indigocarmine.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 23.32 mg of $C_{16}H_8N_2Na_2O_8S_2$

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Indium (^{111}In) Chloride Injection

塩化インジウム (**^{111}In**) 注射液

Indium (^{111}In) Chloride Injection is an aqueous injection.

It contains indium-111 (^{111}In) in the form of indium chloride.

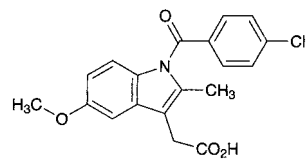
It conforms to the requirements of Indium (^{111}In) Chloride Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Indium (^{111}In) Chloride Injection is a clear, colorless liquid.

Indometacin

インドメタシン



$C_{19}H_{16}ClNO_4$: 357.79
[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid
[*53-86-1*]

Indometacin, when dried, contains not less than 98.0% of indometacin ($C_{19}H_{16}ClNO_4$).

Description Indometacin occurs as a white to light yellow, fine, crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

It is colored by light.

Melting point: 155 – 162°C

It shows crystal polymorphism.

Identification **(1)** Dissolve 2 mg of Indometacin in 100 mL of methanol. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Indometacin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Indometacin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Indometacin RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS with diethyl ether, filter and dry the crystals, and perform the test with the crystals.

(3) Perform the test with Indometacin as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Purity **(1)** Acidity—To 1.0 g of Indometacin add 50 mL of water, shake for 5 minutes, and filter. To the filtrate add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: a red color develops.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Indometacin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Indometacin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Indometacin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dehydrated diethyl ether and acetic acid (100) (100:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Indometacin, previously dried, dissolve in 60 mL of methanol, add 30 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 35.78 mg of $C_{19}H_{16}ClNO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Indometacin Capsules

インドメタシンカプセル

Indometacin Capsules contain not less than 90.0% and not more than 110.0% of the labeled amount of indometacin ($C_{19}H_{16}ClNO_4$: 357.79).

Method of preparation Prepare as directed under Capsules, with Indometacin.

Identification Powder the contents of Indometacin Capsules. To a quantity of the powder, equivalent to 0.1 g of Indometacin, add 20 mL of chloroform, shake well, and centrifuge. Filter the supernatant liquid, and evaporate the filtrate to dryness. After cooling, dissolve the residue in 20 mL of methanol. To 10 mL of this solution add methanol to make 50 mL, then to 2 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 317 nm and 321 nm.

Purity Related substances—Powder the content of Indometacin Capsules. To a quantity of the powder, equivalent to 0.10 g of Indometacin, add exactly 10 mL of methanol, shake well, filter, and use the filtrate as the sample solution. Dissolve 25 mg of Indometacin RS in methanol to make exactly 50 mL. Pipet 1 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Proceed as directed in the Purity (4) under Indometacin.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the content of 1 capsule of Indometacin Capsules, and dissolve in methanol to make exactly V mL so that each mL contains about 1 mg of indometacin ($C_{19}H_{16}ClNO_4$). Filter the solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Indometacin RS, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of indometacin ($C_{19}H_{16}ClNO_4$)
$= M_S \times Q_T/Q_S \times V/25$

M_S: Amount (mg) of Indometacin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of a mixture of water and phosphate buffer solution (pH 7.2) (4:1) as the dissolution medium, the dissolution rate in 20 minutes of Indometacin Capsules is not less than 75%.

Start the test with 1 capsule of Indometacin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 28 μg of indometacin ($C_{19}H_{16}ClNO_4$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Indometacin RS, previously dried at 105°C for 4 hours, dissolve in the dissolution medium to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 320 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of indometacin ($C_{19}H_{16}ClNO_4$)
$= M_S \times A_T/A_S \times 1/C \times 90$

M_S: Amount (mg) of Indometacin RS taken
C: Labeled amount (mg) of indometacin ($C_{19}H_{16}ClNO_4$) in 1 capsule

Assay Weigh accurately the contents of not less than 20 Indometacin Capsules. Powder the combined contents, and weigh accurately a portion of the powder, equivalent to about 50 mg of indometacin ($C_{19}H_{16}ClNO_4$). Dissolve in 40 mL of methanol, and add methanol to make exactly 50 mL. Filter this solution, discarding the first 10-mL portion of the filtrate. Pipet 5 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Indometacin RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of indometacin to that of the internal standard, respectively.

$$\text{Amount (mg) of indometacin } (C_{19}H_{16}ClNO_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Indometacin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol and diluted phosphoric acid (1 in 1000) (7:3).
Flow rate: Adjust so that the retention time of indometacin is about 8 minutes.
System suitability—
System performance: Dissolve 50 mg of 4-chlorobenzoic acid, 30 mg of butyl parahydroxybenzoate and 50 mg of indometacin in 50 mL of methanol. To 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-chlorobenzoic acid, butyl parahydroxybenzoate and indometacin are eluted in this order, with the resolution between the peaks of 4-chlorobenzoic acid and butyl parahydroxybenzoate being not less than 2.0, and between the peaks of butyl parahydroxybenzoate and indometacin being not less than 5.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Indometacin Suppositories

インドメタシン坐剤

Indometacin Suppositories contain not less than 90.0% and not more than 110.0% of the labeled amount of indometacin ($C_{19}H_{16}ClNO_4$: 357.79).

Method of preparation Prepare as directed under Suppositories, with Indometacin.

Identification Dissolve a quantity of Indometacin Suppositories, equivalent to 50 mg of Indometacin, in 20 mL of methanol by warming, add methanol to make 50 mL, and filter if necessary. To 2 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 317 nm and 321 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 suppository of Indometacin Suppositories add 80 mL of a mixture of methanol and acetic acid (100) (200:1), dissolve by warming, add a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL. Pipet V mL of this solution, equivalent to about 2 mg of indometacin ($C_{19}H_{16}ClNO_4$), add a mixture of methanol and acetic acid (100) (200:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Indometacin RS, previously dried at 105°C for 4 hours, and dissolve in a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 320 nm.

$$\text{Amount (mg) of indometacin } (C_{19}H_{16}ClNO_4) = M_S \times A_T/A_S \times 2/V$$

M_S: Amount (mg) of Indometacin RS taken

Assay Weigh accurately not less than 20 Indometacin Suppositories, cut into small pieces carefully, and mix well. Weigh accurately a portion of the mass, equivalent to about 50 mg of indometacin ($C_{19}H_{16}ClNO_4$), add 40 mL of tetrahydrofuran, warm at 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Filter the solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 100 mL. Allow the solution to stand for 30 minutes, filter through a membrane filter (0.5 μm pore size), discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Indometacin RS, previously dried at 105°C for 4 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of the solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of indometacin to that of the internal standard, respectively.

Amount (mg) of indometacin ($C_{19}H_{16}ClNO_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Indometacin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted phosphoric acid (1 in 1000) (7:3).

Flow rate: Adjust so that the retention time of indometacin is about 8 minutes.

System suitability—

System performance: Dissolve 50 mg of 4-chlorobenzoic acid, 30 mg of butyl parahydroxybenzoate and 50 mg of indometacin in 50 mL of methanol. To 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-chlorobenzoic acid, butyl parahydroxybenzoate and indometacin are eluted in this order with the resolution between the peaks of 4-chlorobenzoic acid and butyl parahydroxybenzoate being not less than 2.0 and between the peaks of parahydroxybenzoate and indometacin being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant, and in a cold place.

Influenza HA Vaccine

インフルエンザ **HA** ワクチン

Influenza HA Vaccine is a liquid for injection containing hemagglutinin of influenza virus.

It conforms to the requirements of Influenza HA Vaccine in the Minimum Requirements for Biological Products.

Description Influenza HA Vaccine is a clear liquid or a slightly whitish turbid liquid.

Insulin Glargine (Genetical Recombination)

インスリン　グラルギン（遺伝子組換え）

$C_{267}H_{404}N_{72}O_{78}S_6$: 6062.89
[*160337-95-1*]

Insulin Glargine (Genetical Recombination) is an analogue of human insulin (genetical recombination), being substituted asparagine residue with glycine residue at 21st of A chain and added two arginine residues at C-terminal of B chain. It is a peptide composed with A chain consisting of 21 amino acid residues and B chain consisting of 32 amino acid residues, and has an activity to reduce the blood glucose level.

It contains not less than 94.0% and not more than 105.0% of insulin glargine (genetical recombination) ($C_{267}H_{404}N_{72}O_{78}S_6$), calculated on the anhydrous basis.

0.0364 mg of Insulin Glargine (Genetical Recombination) is equivalent to 1 Insulin Unit.

Description Insulin Glargine (Genetical Recombination) occurs as a white powder.

It is practically insoluble in water and in ethanol (99.5).

It is sparingly soluble in 0.01 mol/L hydrochloric acid TS.

It is hygroscopic.

It is gradually decomposed by light.

Identification Keep the sample solution and standard solution at 2 – 8°C. Weigh a suitable amount of Insulin Glargine (Genetical Recombination) and Insulin Glargine RS, and dissolve separately in 0.01 mol/L hydrochloric acid TS so that each mL contains 10.0 mg. Transfer 5 μL of these solutions into clean test tubes, add 1 mL of 1 mol/L tris buffer solution (pH 7.5) and 100 μL of a solution of V8 protease for insulin glargine in 1 mol/L tris buffer solution (pH 7.5) (20 units/mL), allow to react at 35 – 37°C for 3 hours, then add 2 μL of phosphoric acid to stop the reaction, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from these solutions: The similar peaks appear at the same retention times.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 3 mm in inside diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: To 930 mL of a solution, prepared by dissolving 11.6 g of phosphoric acid and 42.1 g of sodium perchloric acid in 1600 mL of water, adjusting to pH 2.3 with triethylamine and adding water to make 2000 mL, add 70 mL of acetonitrile for liquid chromatography.

Mobile phase B: To 430 mL of a solution, prepared by dissolving 11.6 g of phosphoric acid and 42.1 g of sodium perchloric acid in 1600 mL of water, adjusting to pH 2.3 with

triethylamine and adding water to make 2000 mL, add 570 mL of acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as follows.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	90 → 20	10 → 80
30 – 35	20	80

Flow rate: 0.55 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the symmetry factors of the two larger peaks, which appear next to the first peak just after the solvent peak, are not more than 1.5, respectively, and the resolution between these peaks is not less than 3.4.

Purity **(1)** Related substances—Perform the test with 5 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of these peaks by the area percentage method: the amount of the peak other than insulin glargine is not more than 0.4%, and the total amount of the peaks other than insulin glargine is not more than 1.0%.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: For 40 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the peak area of insulin glargine obtained with 5 μL of this solution is equivalent to 5 to 15% of that obtained with 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of insulin glargine are not less than 20,000 and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

(2) High-molecular mass proteins—Keep the sample solution at 2 – 8°C. Dissolve 15 mg of Insulin Glargine (Genetical Recombination) in 1.5 mL of 0.01 mol/L hydrochloric acid TS, add water to make 10 mL, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than insulin glargine is not more than 0.3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 276 nm).

Column: Two stainless steel columns connected in series of 8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 400 mL of water add 300 mL of acetonitrile for liquid chromatography and 200 mL of acetic acid (100), adjust to pH 3.0 with ammonia solution (28), and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of insulin glargine is about 35 minutes.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the completion of the elution of insulin glargine.

System suitability—

Test for required detectability: To 1 mL of the sample solution add 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the peak area of insulin glargine obtained with 100 μL of this solution is equivalent to 5 to 15% of that obtained with 100 μL of the solution for system suitability test.

System performance: Heat 15 mg of Insulin Glargine (Genetical Recombination) at 100°C for 1.5 – 3 hours, then dissolve in 1.5 mL of 0.01 mol/L hydrochloric acid TS, and add water to make exactly 10 mL. When the procedure is run with 100 μL of this solution under the above operating conditions, the high-molecular mass protein and insulin glargine are eluted in this order with the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 100 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

(3) Other product-related impurities—Being specified separately when the drug is granted approval based on the Law.

(4) Host cell proteins—Being specified separately when the drug is granted approval based on the Law.

(5) DNA—Being specified separately when the drug is granted approval based on the Law.

Water <*2.48*> Not more than 8.0% (90 mg, coulometric titration).

Bacterial endotoxins <*4.01*> Less than 10 EU/mg.

Zinc content Weigh accurately about 45 mg of Insulin Glargine (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make three solutions containing 0.20 μg, 0.40 μg and 0.60 μg of zinc (Zn: 65.38) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of zinc in

the sample solution using a calibration curve obtained from the absorbances of the standard solutions: not more than 0.80% of zinc (Zn: 65.38), calculated on the anhydrous basis.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow-cathode lamp.
Wavelength: 213.9 nm.

Assay Keep the sample solution and standard solution at 2 – 8°C. Weigh accurately about 15 mg of Insulin Glargine (Genetical Recombination), dissolve in 1.5 mL of 0.01 mol/L hydrochloric acid TS, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve Insulin Glargine RS in 0.01 mol/L hydrochloric acid TS so that each mL contains about 10 mg of insulin glargine, then exactly dilute with water so that each mL contains about 1.5 mg of insulin glargine, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of insulin glargine in each solution.

Amount (mg) of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of insulin glargine in 1 mL of the standard solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 3 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 20.7 g of anhydrous sodium dihydrogen phosphate in 900 mL of water, adjust to pH 2.5 with phosphoric acid, and add water to make 1000 mL. To 250 mL of this solution add 250 mL of acetonitrile for liquid chromatography, dissolve 18.4 g of sodium chloride in this solution, and add water to make 1000 mL.

Mobile phase B: Dissolve 20.7 g of anhydrous sodium dihydrogen phosphate in 900 mL of water, adjust to pH 2.5 with phosphoric acid, and add water to make 1000 mL. To 250 mL of this solution add 650 mL of acetonitrile for liquid chromatography, dissolve 3.2 g of sodium chloride in this solution, and add water to make 1000 mL.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	96 → 83	4 → 17
20 – 30	83 → 63	17 → 37
30 – 40	63 → 96	37 → 4

Flow rate: 0.55 mL per minute (the retention time of insulin glargine is about 21 minutes).

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of insulin glargine are not less than 20,000 and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

Containers and storage Containers—Tight containers.
Storage—Not exceeding −15°C.

Insulin Glargine (Genetical Recombination) Injection

インスリン　グラルギン(遺伝子組換え)注射液

Insulin Glargine (Genetical Recombination) Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled Insulin Unit of insulin glargine (genetical recombination) ($C_{267}H_{404}N_{72}O_{78}S_6$: 6062.89).

Method of preparation Prepare as directed under Injections, with Insulin Glargine (Genetical Recombination).

Description Insulin Glargine (Genetical Recombination) Injection occurs as a clear, colorless liquid.

Identification (1) Insulin Glargine (Genetical Recombination) Injection forms a precipitate when adjusted to pH 5.7 – 6.5 with dilute sodium hydroxide TS, and the precipitate disappears when adjusted to pH 3.5 – 4.5 with 0.1 mol/L hydrochloric acid TS.

(2) Perform the test with 5 μL each of the sample solution and the standard solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the conditions described in the Assay: the retention times of the principal peaks obtained from the sample solution and standard solution are the same.

pH Being specified separately when the drug is granted approval based on the Law.

Purity (1) Related substances—Keep the sample solution at 2 – 8°C. Perform the test with 5 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak other than insulin glargine is not more than 0.5%, and the total amount of the peaks other than insulin glargine is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Insulin Glargine (Genetical Recombination).

Time span of measurement: For 40 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the peak area of insulin glargine obtained with 5 μL of this solution is equivalent to 5 to 15% of that obtained with 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the standard solution obtained in the Assay under the

above operating conditions, the number of theoretical plates and the symmetry factor of the peak of insulin glargine are not less than 20,000 and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

(2) High-molecular mass proteins—To a suitable amount of Insulin Glargine (Genetical Recombination) Injection add water so that each mL contains 40 Insulin Units, and use this solution as the sample solution. Then, proceed as directed in the Purity (2) under Insulin Glargine (Genetical Recombination).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Zinc content Being specified separately when the drug is granted approval based on the Law.

Assay To a suitable amount of Insulin Glargine (Genetical Recombination) Injection add exactly water so that each mL contains 40 Insulin Units, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Glargine (Genetical Recombination).

Amount (Insulin Unit) of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$) in 1 mL

$= M_S \times A_T/A_S \times d \times 1/0.0364$

M_S: Amount (mg) of insulin glargine in 1 mL of the standard solution

d: Dilution factor of the sample solution

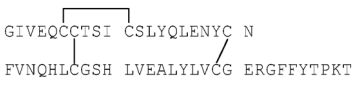
0.0364: Mass (mg) of insulin glargine equivalent to 1 Insulin Unit

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, at a temperature of 2 – 8°C avoiding freezing.

Insulin Human (Genetical Recombination)

インスリン ヒト(遺伝子組換え)



$C_{257}H_{383}N_{65}O_{77}S_6$: 5807.57

[*11061-68-0*]

Insulin Human (Genetical Recombination) is a recombinant human insulin. It is a peptide composed of A chain consisting of 21 amino acid residues and B chain consisting of 30 amino acid residues, and has an activity to reduce the blood glucose level.

It contains not less than 27.5 Insulin Units per mg, calculated on the dried basis.

Description Insulin Human (Genetical Recombination) occurs as a white powder.

It is practically insoluble in water and in ethanol (95).

It dissolves in 0.01 mol/L hydrochloric acid TS and in sodium hydroxide TS with decomposition.

It is hygroscopic.

Identification Weigh accurately a suitable amount of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg. Transfer 500 μL of this solution into a clean test tube, add 2.0 mL of HEPES buffer solution (pH 7.5) and 400 μL of V8-protease TS, incubate at 25°C for 6 hours, then add 2.9 mL of ammonium sulfate buffer solution to stop the reaction, and use this solution as the sample solution. Separately, proceed with Insulin Human RS in the same manner as above, and use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and compare the chromatograms obtained from these solutions: a similar peak is observed at the same retention time in the both chromatograms.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of water, ammonium sulfate buffer solution and acetonitrile (7:2:1).

Mobile phase B: A mixture of water, acetonitrile and ammonium sulfate buffer solution (2:2:1).

Flowing of mobile phase: Change the mixing ratio of the mobile phase A and B linearly from 9:1 to 3:7 in 60 minutes after sample injection, further change to 0:10 linearly in 5 minutes, and then flow the mobile phase B only for 5 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the symmetry factor of the two larger peaks which appear next to the first peak just after the solvent peak are not more than 1.5 respectively, and the resolution between these peaks is not less than 3.4.

Purity **(1)** Related substances—Perform this procedure rapidly. Dissolve 7.5 mg of Insulin Human (Genetical Recombination) in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak area of human insulin, A_1, the peak area of the desamido substance having the relative retention time of about 1.3 to human insulin, A_D, and the total area of the peaks other than the solvent peak, A_T: the amounts of the desamido substance and related substances other than the desamido substance are each not more than 2.0%. Previously, perform the test with 0.01 mol/L hydrochloric acid TS in the same manner to confirm the solvent peak.

Amount (%) of the desamido substance $= A_D/A_T \times 100$

Amount (%) of related substances other than the desamido substance

$= [\{A_T - (A_1 + A_D)\}/A_T] \times 100$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of phosphoric acid-sodium sulfate buffer solution (pH 2.3) and acetonitrile for liquid chromatography (41:9).

Mobile phase B: A mixture of phosphoric acid-sodium sulfate buffer solution (pH 2.3) and acetonitrile for liquid chromatography (1:1).

Flowing of mobile phase: Flow a mixture of the mobile phase A and B (78:22) for 36 minutes before and after the sample injection, then change the mixing ratio to 33:67 linearly in 25 minutes, and maintain this ratio for 6 minutes. Then flow the first mixture (78:22) for the next 15 minutes. Adjust the mixing ratio of the first mixture so that the retention time of human insulin is about 25 minutes.

Flow rate: 1.0 mL per minute.

Time span of measurement: For about 75 minutes after the sample is injected.

System suitability—

Test for required detectability: Confirm that the peak height of the desamido substance obtained from 20 μL of human insulin desamido substance-containing TS is between 30% and 70% of the full scale.

System performance: When the procedure is run with 20 μL of human insulin desamido substance-containing TS under the above operating conditions, human insulin and human insulin desamido substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.

(2) High-molecular mass proteins—Dissolve 4 mg of Insulin Human (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 100 μL of this solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate each peak area: the total of areas of the peaks having smaller retention time than human insulin is not more than 1.0% of the total area of all peaks.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 276 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of L-arginine (1 in 1000), acetonitrile and acetic acid (100) (13:4:3).

Flow rate: Adjust so that the retention time of human insulin is about 20 minutes.

Time span of measurement: Until the peak of human insulin monomer has appeared.

System suitability—

Test for required detectability: Confirm that the peak height of the dimer obtained from 100 μL of human insulin dimer-containing TS is between 10% and 50% of the full scale.

System performance: When the procedure is run with 100 μL of human insulin dimer-containing TS under the above operating conditions, polymer, dimer and monomer are eluted in this order, and the ratio, H_1/H_2, of the peak height of the dimer H_1 to the height of the bottom between the peaks of the dimer and the monomer H_2 is not less than 2.0.

(3) Product related impurities—Being specified separately when the drug is granted approval based on the Law.

(4) Process related impurities—Being specified separately when the drug is granted approval based on the Law.

Loss on drying <*2.41*> Not more than 10.0% (0.2 g, 105°C, 24 hours).

Bacterial endotoxins <*4.01*> Less than 10 EU/mg.

Zinc content Weigh accurately about 50 mg of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL. If necessary, dilute with 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains between 0.4 μg and 1.6 μg of zinc (Zn: 65.38), and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make solutions containing 0.40 μg, 0.80 μg, 1.20 μg and 1.60 μg of zinc (Zn: 65.38) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*>, and calculate the amount of zinc (Zn: 65.38) in the sample solution by using a calibration curve obtained from the absorbances of the standard solutions: not more than 1.0%, calculated on the dried basis.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.

Assay Perform this procedure quickly. Weigh accurately about 7.5 mg of Insulin Human (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately a suitable amount of Insulin Human RS, dissolve exactly in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 40 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of human insulin, A_{TI} and A_{SI}, and the peak areas of the desamido substance having the relative retention time of 1.3 to human insulin, A_{TD} and A_{SD}, respectively, of these solutions.

Amount (Insulin Unit/mg) of human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$)
$= (M_S \times F)/D \times (A_{TI} + A_{TD})/(A_{SI} + A_{SD}) \times 5/M_T$

F: Labeled unit (Insulin Unit/mg) of Insulin Human RS
D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve the reference standard
M_T: Amount (mg) of Insulin Human (Genetical Recombination) taken, calculated on the dried basis
M_S: Amount (mg) of Insulin Human RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphoric acid-sodium sulfate buffer solution (pH 2.3) and acetonitrile for liquid chromatography (3:1). Adjust the mixing ratio of the component of the mobile phase so that the retention time of human insulin is between 10 minutes and 17 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of human insulin desamido substance-containing TS under the above operating conditions, human insulin and human insulin desamido substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of human insulin is not more than 1.6%.

Containers and storage Containers—Tight containers.

Storage—Not exceeding −20°C.

Insulin Human (Genetical Recombination) Injection

インスリン ヒト(遺伝子組換え)注射液

Insulin Human (Genetical Recombination) Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled Insulin Unit of insulin human (genetical recombination) ($C_{257}H_{383}N_{65}O_{77}S_6$: 5807.57).

Method of preparation Prepare as directed under Injections, with Insulin Human (Genetical Recombination) suspended in Water for Injection then dissolved by addition of Hydrochloric Acid or Sodium Hydroxide.

Description Insulin Human (Genetical Recombination) Injection occurs as a clear, colorless liquid, and slightly a fine precipitate may be observable upon storage.

Identification Insulin Human (Genetical Recombination) Injection forms a precipitate when adjusted to pH 5.3 – 5.5 by addition of dilute hydrochloric acid, and the precipitate disappears when adjusted to pH 2.5 – 3.5 by further addition of the acid.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH Being specified separately when the drug is granted approval based on the Law.

Purity (1) Desamido substance—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak, having the relative retention time of about 1.3 to human insulin, is not more than 1.5%.

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Insulin Human (Genetical Recombination).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay under Insulin Human (Genetical Recombination).

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of human insulin obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 20 μL of the sample solution.

System repeatability: Dissolve Insulin Human RS in 0.01 mol/L hydrochloric acid TS so that each mL contains about 4 Insulin Units. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of human insulin is not more than 2.0%.

(2) High-molecular mass proteins—For each mL of Insulin Human (Genetical Recombination) Injection add 4 μL of 6 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than human insulin is not more than 2.0%.

Operating conditions—

Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Insulin Human (Genetical Recombination).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the completion of the elution of human insulin.

System suitability—

System performance: Proceed as directed in the system suitability in the Purity (2) under Insulin Human (Genetical Recombination).

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of human insulin obtained with 100 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 100 μL of the sample solution.

Bacterial endotoxins <*4.01*> Less than 0.80 EU/Insulin Unit. Apply to the preparations intended for intravenous administration.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Zinc content To an exact volume of Insulin Human (Genetical Recombination) Injection, equivalent to 300 Insulin Units, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. If necessary, further dilute with 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make three solutions containing 0.20 μg, 0.60 μg and 1.20 μg of zinc (Zn: 65.38) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotome-

try <2.23> according to the following conditions, using the 0.01 mol/L hydrochloric acid TS as the blank, and calculate the amount of zinc in the sample solution by using a calibration curve obtained from the absorbances of the standard solutions: 10 – 40 μg of zinc (Zn: 65.38) per 100 Insulin Units.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.

Assay To exactly 10 mL of Insulin Human (Genetical Recombination) Injection add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Human (Genetical Recombination).

Amount (Insulin Unit) of human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL

$= M_S \times F/D \times (A_{T1}+A_{TD})/(A_{S1}+A_{SD}) \times 1.004 \times 5/2$

M_S: Amount (mg) of Insulin Human RS taken
F: Labeled unit (Insulin Unit/mg) of Insulin Human RS
D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve Insulin Human RS

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, at a temperature of 2 – 8°C avoiding freezing.

Interferon Alfa (NAMALWA)

インターフェロン　アルファ(NAMALWA)

Interferon Alfa (NAMALWA) is essentially a human interferon alfa, which is a glycoprotein (molecular mass: 17,000 – 30,000) produced by the human lymphoblast NAMALWA cell induced by Sendai virus. It is an aqueous solution. It possesses the antiviral activity.

It contains not less than 50 μg and not more than 500 μg of protein per mL, and not less than 1.0×10^8 Units per mg of the protein.

Description Interferon Alfa (NAMALWA) occurs as a clear and colorless liquid.

Identification **(1)** To Interferon Alfa (NAMALWA) add Eagle's minimum essential medium containing bovine serum so that each mL contains 5000 Units, and use this solution as the sample stock solution. To anti-interferon alfa antiserum add an amount of Eagle's minimum essential medium containing bovine serum so that each mL contains an amount of anti-interferon alfa antiserum which neutralizes 10,000 Units of interferon alfa. To this solution add an equal volume of the sample stock solution, stir, and use this solution as the sample solution. Separately, to the sample stock solution add an equal volume of Eagle's minimum essential medium containing bovine serum, stir, and use this solution as the control solution. Determine the remained potency of the sample solution and control solution after allowing to stand at 37 ± 1°C for 1 hour, according to the Assay. When the antiviral activity of Interferon Alfa (NAMALWA) is neutralized by anti-interferon alfa antiserum, it meets the requirement. Not detection of the remaining potency of the sample solution is a criterion of neutralization.

(2) Soak polyvinylidene fluoride membrane in methanol for 10 – 20 seconds, then soak additionally in phosphate-buffered sodium chloride TS for more than 30 minutes. To the well in the dot blot apparatus mounted the polyvinylidene fluoride membrane, add a volume of Interferon Alfa (NAMALWA), corresponding to about 20 μg protein, allow to stand for 15 minutes, and aspirate. After repeating twice to aspirate with a 0.2-mL portion of phosphate-buffered sodium chloride TS, take out the polyvinylidene fluoride membrane, soak in 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4), and stir gently for 10 minutes. Replace the liquid, and repeat this operation two more times. Remove 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4), add elderberry lectin TS, and stir gently for 2 hours. Remove the elderberry lectin TS, add 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4), and stir gently for 10 minutes. Replace the liquid, and repeat this operation two more times. Remove 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4), add the peroxidase-labeled avidin TS, and stir gently for 15 minutes. Remove the peroxidase-labeled avidin TS, add 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4), and stir gently for 10 minutes. Replace the liquid, and repeat this operation two more times. Remove 0.01 mol/L tris buffer solution-sodium chloride TS (pH 7.4), add substrate TS for interferon alfa identification, and allow to develop the color: a brown dot is observed.

Constituent amino acids When perform the test by Method 2 of 2. Methodologies of Amino Acid Analysis after hydrolyzing by Method 1 (but not containing phenol) of 1. Hydrolysis of Protein and Peptide under Amino Acid Analysis of Proteins <2.04>, the molar ratios of each constituent amino acid are 8 – 11 for aspartic acid, 4 – 7 for threonine, 7 – 10 for serine, 16 – 19 for glutamic acid, 2 – 4 for glycine and tyrosine, 5 – 7 for alanine, phenylalanine and lysine, 3 – 6 for valine, 2 – 5 for methionine, 4 – 6 for isoleucine, 12 – 15 for leucine, 1 – 3 for histidine and 6 – 9 for arginine.

(i) Hydrolysis—To Interferon Alfa (NAMALWA) add tris-glycine buffer solution (pH 6.8) so that each mL contains 6,000,000 Units. Pass 3 mL of the solution through a column 4 mm in internal diameter, packed with 0.145 g of ethylsilanized silica gel for column chromatography and previously washed with 5 mL of a mixture of water, acetonitrile and diluted trifluoroacetic acid (1 in 50) (13:6:1). Then, after washing with not less than 10 mL of a mixture of water, acetonitrile and diluted trifluoroacetic acid (1 in 50) (13:6:1), elute interferon alfa with 0.5 mL of a mixture of acetonitrile and diluted trifluoroacetic acid (1 in 50) (19:1), and use the eluate as the sample stock solution. To 0.45 mL of the sample stock solution add 50 μL of the internal standard solution, and stir. Transfer 0.1 mL each of this solution into two glass vessels for hydrolysis, and evaporate to dryness under reduced pressure. Add 20 μL of a solution which is prepared by adding 10 μL of mercapto acetic acid to 1 mL of 6 mol/L hydrochloric acid TS for amino acid automatic analysis, and 0.18 mL of 6 mol/L hydrochloric acid TS for amino acid automatic analysis to the bottom of the glass vessels, replace the air in the vessels with nitrogen, close the vessels tightly under reduced pressure, and heat at 110 ± 2°C for 24 hours for one of the vessels and for 72 hours for another. After cooling, open the vessels, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in 20 μL of water, and evaporate to dryness under reduced pressure. Dissolve the residues with 0.1 mL each of diluted 6 mol/L hydrochloric acid TS for amino acid automatic analysis (31 in 10,000), and use these solutions as the sample solutions (1) and (2), respectively. Separately, weigh exactly a suitable amount each of L-lysine hydrochloride, L-histidine hydro-

chloride monohydrate, L-arginine, L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine and L-norleucine, dissolve in diluted 6 mol/L hydrochloric acid TS for amino acid automatic analysis (31 in 10,000) so that each mL contains a certain concentration of about 20 nmol for each amino acid, and use this solution as the standard solution.

(ii) Amino acid analysis—When perform the test with 15 μL each of the sample solutions (1) and (2) and 10 μL of the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, either chromatogram obtained from the sample solutions shows the peaks corresponding to the peaks obtained from the standard solution. The molar ratios of each constituent amino acids are calculated. When calculate the molar ratios of each constituent amino acid, for threonine and serine the molar value is corrected by extrapolation to 0 hour-heating based on the values obtained from the sample solutions (1) and (2), for isoleucine and valine use the value obtained from the sample solution (2), and for the other amino acids use the value obtained from the sample solution (1). The molar ratios of cystine, proline and tryptophan are excluded from calculation.

Internal standard solution—To exactly 32.81 mg of L-norleucine add diluted 6 mol/L hydrochloric acid TS for amino acid automatic analysis (31 in 10,000) to make exactly 100 mL. Pipet 4 mL of this solution, add diluted 6 mol/L hydrochloric acid TS for amino acid automatic analysis (31 in 10,000) to make exactly 100 mL.

Operating conditions—

Detector: A fluorophotometer (excitation wavelength: 340 nm, fluorescence wavelength: 450 nm).

Column: A stainless steel column 5 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin (Na type) for liquid chromatography composed with a sulfonated styrene-divinylbenzene copolymer (3 μm in particle diameter).

Column temperature: Inject the sample at 50 ± 1°C, maintain the temperature for 11 minutes, change to 40 ± 1°C and maintain for 23 minutes, then change to 65 ± 1°C and maintain for 56 minutes, and change to 45 ± 1°C.

Reaction vessel temperature: A constant temperature of about 51°C.

Mobile phase: Prepare the mobile phases A, B, C and D according to the following table.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D
Citric acid monohydrate	15.93 g	8.40 g	6.10 g	—
Sodium citrate hydrate	6.97 g	10.00 g	26.67 g	—
Sodium chloride	6.36 g	2.34 g	54.35 g	—
Sodium hydroxide	—	—	2.0 g	8.0 g
Ethanol (99.5)	54 mL	—	—	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL
Benzyl alcohol	—	2 mL	5 mL	—
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Water	a suitable quantity	a suitable quantity	a suitable quantity	a suitable quantity
Total amount	1000 mL	1000 mL	1000 mL	1000 mL

Flowing of mobile phase: Control the gradient by mixing the mobile phases A, B, C and D as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)
0 – 11	100	0	0	0
11 – 12	100 → 0	0 → 100	0	0
12 – 34	0	100	0	0
34 – 39.1	0	100 → 0	0 → 100	0
39.1 – 71	0	0	100	0
71 – 86	0	0	0	100

Reaction reagent: Prepare the reaction reagents A, B and C according to the following table.

	Reaction reagent A	Reaction reagent B	Reaction reagent C
Sodium hydroxide	24.0 g	—	—
Boric acid	—	21.60 g	21.60 g
o-Phthalaldehyde in ethanol (99.5) solution (2 in 25)	—	—	10 mL
Lauromacrogol solution (1 in 4)	—	—	4 mL
2-Mercaptoethanol	—	—	2 mL
10% Sodium hypochlorite TS	—	0.1 mL	—
Water	a suitable quantity	a suitable quantity	a suitable quantity
Total amount	1000 mL	1000 mL	1000 mL

Flow rate of mobile phase: Adjust so that the retention times of aspartic acid, glutamic acid and methionine are about 12, 20 and 42 minutes, respectively.

Flow rate of reaction reagent: About 0.2 mL per minute for each of reagent A, B, and C.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine are not less than 0.6, not less than 0.8 and not less than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of aspartic acid, proline, valine, and arginine are not more than 2.5%, respectively.

Molecular mass To a suitable amount of Interferon Alfa (NAMALWA) add tris-glycine buffer solution (pH 6.8) so that each mL contains 6,000,000 Units. To 3 volumes of this solution add 1 volume of reduction liquid for molecular mass determination, heat on a water bath for 90 seconds, and use this solution as the sample solution. Separately, to 3 volumes of molecular mass marker for interferon alfa add 1 volume of reduction liquid for molecular mass determination, heat on a water bath for 90 seconds, and use this solution as the standard solution. After performing the electrophoresis with 40 μL of the sample solution and 15 μL of the standard solution using tris buffer solution (pH 8.3) and polyacrylamide gel for interferon alfa, fix the gel by immersing for 1 hour in a solution of trichloroacetic acid (3 in 20). Then, stain the gel by immersing for more than 2 hours in a solution, prepared by dissolving 1.0 g of Coomassie brilliant blue R-250 in 450 mL of methanol and 100 mL of acetic acid (100) and adding water to make 1000 mL, and destain by immersing the gel in 1000 mL of a mixture of water, methanol and acetic acid (100) (33:4:3). Determine the relative mobility of each band obtained from the standard solution, and prepare a calibration curve by linear regression against the

logarithm of molecular mass. Determine the relative mobility of the center of the main band obtained from the sample solution, and calculate the molecular mass of Interferon Alfa (NAMALWA) from the calibration curve: at least 4 bands are observed between 17,000 and 30,000 of molecular mass.

Purity **(1)** Egg albumin, Sendai virus coat protein, other foreign proteins, and other process-related impurities—Being specified separately when the drug is granted approval based on the Law.

(2) Nucleic acids—Perform the test according to the following method: the amount of nucleic acids is not more than 1.0 pg as DNA per 1,000,000 Units of interferon alfa (NAMALWA).

(i) DNA standard solutions: To the DNA standard stock solution for interferon alfa (NAMALWA) add salmon sperm DNA solution (1 in 10,000,000) so that each mL contains exactly 20 ng DNA. Hereinafter, the concentration of DNA is the concentration of DNA for interferon alfa (NAMALWA). To this solution add tris-glycine buffer solution (pH 6.8) exactly so that each mL contains 10 ng DNA. Then, dilute serially by adding tris-glycine buffer solution (pH 6.8). Dilute exactly with a mixture of tris-glycine buffer solution (pH 6.8) and 1 mol/L tris buffer solution (pH 8.0) (40:1) so that each mL contains 128, 64, 32, 16, 8, and 4 pg of DNA, respectively, and use these solutions as DNA standard solutions.

(ii) Procedure: Use Interferon Alfa (NAMALWA) as the sample solution. Place 0.11 mL each of DNA standard solutions, a mixture of tris-glycine buffer solution (pH 6.8) and 1 mol/L tris buffer solution (pH 8.0) (43:1), and the sample solution into tubes separately. Heat these solutions in an aluminum block thermostat bath at 98°C for 10 minutes. After ice-cooling, centrifuge, and transfer 50 μL each of the supernatants to new tubes. In separate wells of a PCR microplate place 6 μL each of DNA standard solutions which have been treated by heating for DNA extraction, a mixture of tris-glycine buffer solution (pH 6.8) and 1 mol/L tris buffer solution (pH 8.0) (43:1), and the sample solution. Then, add 20 μL each of a mixture of 2-fold PCR reaction solution containing SYBR Green, nuclease free water, primer F TS and primer R TS (167:70:10:10) into each well. Seal with plate film, and centrifuge. After centrifugation, attach the plate to a real-time PCR system, repeat 40 cycles of 15 seconds at 95°C and 1 minute at 60°C, and measure the fluorescence intensity of each well in every PCR cycle. Plot the fluorescent amount on the vertical axis and the PCR cycle number on the horizontal axis, and determine the PCR cycle number at which the fluorescence of each well is greater than a certain value. Further, make a calibration curve by plotting the number of PCR cycles on the vertical axis and the logarithm of the concentration of DNA standard solution on the horizontal axis to calculate the concentration of DNA in the sample solution.

System suitability—

Test for required detectability: The PCR cycle number obtained with 4 pg/mL DNA standard solution is not greater than that obtained with a mixture of tris-glycine buffer solution (pH 6.8) and tris buffer solution (pH 8.0) (43:1).

System performance: When the procedure is run with each DNA standard solution under the above conditions, the correlation coefficient of the calibration curve obtained is 0.990 or more.

(3) Infective virus test—Inject 0.2 mL each of Interferon Alfa (NAMALWA) into the allantoic cavity of not less than 6 embryonated eggs, allow them to stand at 36 ± 1°C for 3 days, and then allow to stand at 4°C for a night. Collect more than 1 mL of the allantoic fluid from each egg. To 50 μL of the allantoic fluid add 50 μL of 0.5 vol% chicken erythrocyte suspension, mix, and allow to stand at room temperature for 1 hour. Examine the presence of the aggregation. When the aggregation is not found, inject 0.2 mL each of this allantoic fluid into the allantoic cavity of the embryonated eggs, and repeat the same procedure as above: the test is met when the aggregation is not found. As a positive control, inoculate the Sendai virus 1.6×10^{-4} to 6.4×10^{-4} HA value per embryonated chicken egg into the allantoic cavity, and perform the test at the same time.

Assay **(1)** Protein content—

(i) Sample solution: Dilute Interferon Alfa (NAMALWA) with isotonic sodium chloride solution so that each mL contains 3,000,000 to 4,000,000 Units, and use this solution as the sample solution.

(ii) Standard solution: Weigh accurately about 50 mg of bovine serum albumin, and dissolve in isotonic sodium chloride solution to make exactly 50 mL. Determine the absorbance of this solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Calculate the protein concentration based on $E^{1\%}_{1\,cm}$ (280 nm) = 6.6. To this solution add isotonic sodium chloride solution so that each mL contains exactly 50, 25, 12.5, 6.25, and 3.13 μg of the bovine serum albumin, and use these solutions as the standard solutions.

(iii) Procedure: To exactly 0.25 mL each of the sample solution and the standard solutions add exactly 0.25 mL of Coomassie brilliant blue TS for interferon alfa, and allow to stand at room temperature for exactly 30 seconds. Determine the absorbance of these solutions at 614 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank. Plot the absorbance of the standard solutions on the vertical axis and their protein concentrations on the horizontal axis to prepare a calibration curve. Determine the protein content of the sample solution from its absorbance using the calibration curve, and calculate the amount of protein per mL of the sample solution. Perform a blank determination in the same manner with isotonic sodium chloride solution, and make any necessary correction.

(2) Specific activity—To each well of a flat-bottom microplate add 45,000 to 60,000 cells of FL cell, prepared with Eagle's minimum essential medium containing bovine serum, and incubate at 37 ± 1°C for 18 to 22 hours in an incubator filled with 5% carbon dioxide. Dilute Interferon Alfa (NAMALWA) and Interferon Alfa RS separately with Eagle's minimum essential medium containing bovine serum so that each mL contains about 30 Units, and use these solutions as the sample solution (1) and the standard solution (1), respectively. To 200 μL each of these solutions add 117 μL of Eagle's minimum essential medium containing bovine serum, and use these solutions as the sample solution (2) and the standard solution (2), respectively. Repeat this operation, and prepare the sample solutions and standard solutions with log dilutions of 8 serials (dilution ratio per stage is 0.2 $\log_{10}$ fold). Repeat to prepare the sample solutions three or more times. Add each sample solution or standard solution into each well of the cell culture, and incubate at 37 ± 1°C for 6 hours. Discard the culture medium, add 1×10^5 to 1×10^6 PFU of Sindbis virus per well, and incubate at 37 ± 1°C for 38 to 42 hours. Discard the culture medium, add neutral red-Eagle's minimum essential medium containing bovine serum, and incubate at 37 ± 1°C for 45 to 75 minutes. Discard the culture medium, and add 0.01 mol/L phosphate buffer solution. Discard the liquid. Repeat this

operation. Elute the neutral red that is taken up by the cells by adding sodium dihydrogen phosphate-ethanol TS. Determine the absorbance at 540 nm, prepare the dose-response curves by plotting the absorbances on the vertical axis and the logarithm of the dilution ratio on the horizontal axis with the absorbances obtained from the sample solution and standard solution. On the dose-response curves of the sample solution and standard solution, calculate the relative potency of the sample solution (n = 3 or more), obtained independently, to the standard solution by comparing the points where the intermediate of absorbances in cells infected with virus and cells not infected with virus, and calculate the average value of them as the potency of Interferon Alfa (NAMALWA) in 1 mL. Calculate the specific activity by dividing the obtained potency by the amount of protein content.

When all of the following conditions are satisfied, the test is valid.

Absorbance obtained from cells not infected with virus is 0.8 to 1.2.

Absorbance obtained from the cells infected with virus is not more than 0.1.

Standard deviation of the (log) potency of Interferon Alfa (NAMALWA) in 1 mL obtained from the sample solution prepared three or more times independently is not more than 0.06.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C, avoiding freezing.

Interferon Alfa (NAMALWA) Injection

インターフェロン　アルファ(NAMALWA)注射液

Interferon Alfa (NAMALWA) Injection is an aqueous injection.

It contains not less than 70% and not more than 150% of the labelled amount of interferon alfa (NAMALWA).

Method of preparation Prepare as directed under Injections, with Interferon Alfa (NAMALWA).

Description Interferon Alfa (NAMALWA) Injection is a clear and colorless liquid.

Identification To Interferon Alfa (NAMALWA) Injection add Eagle's minimum essential medium containing bovine serum so that each mL contains 5000 Units, and use this solution as the sample stock solution. To anti-interferon alfa antiserum add an amount of Eagle's minimum essential medium containing bovine serum so that each mL contains an amount of anti-interferon alfa antiserum which neutralizes 10,000 Units of interferon alfa. To this solution add an equal volume of the sample stock solution, stir, and use this solution as the sample solution. Separately, to the sample stock solution add an equal volume of Eagle's minimum essential medium containing bovine serum, stir, and use this solution as the control solution. Determine the remained potency of the sample solution and control solution after allowing to stand at 37 ± 1°C for 1 hour, according to the Assay. When neutralized the antiviral activity of Interferon Alfa (NAMALWA) by anti-interferon alfa antiserum, it meets the requirement. Not detection of the remaining potency of the sample solution is the criterion of neutralization.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Multimers—To a suitable amount of Interferon Alfa (NAMALWA) Injection add tris-glycine buffer solution (pH 6.8) so that each mL contains 3,000,000 Units, and use this as the sample solution. Perform the test with 200 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the total amount of the peaks, having the retention time smaller than that of interferon alfa monomer, is not more than 3.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A glass column 10 mm in inside diameter and 30 cm in length, packed with dextran-highly cross-linked agarose gel filtration carrier for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.15 g of anhydrous disodium hydrogen phosphate, 0.2 g of potassium dihydrogen phosphate, 8.0 g of sodium chloride and 0.2 g of potassium chloride in water to make 1000 mL. To 950 mL of this solution add 50 mL of a solution prepared by dissolving 10 g of sodium lauryl sulfate in 100 mL of water, and mix gently.

Flow rate: 1 mL per minute.

Time span of measurement: Until the elution of interferon alfa monomer is completed.

System suitability—

Test for required detectability: Pipet 50 μL of the sample solution, add tris-glycine buffer solution (pH 6.8) to make exactly 2 mL. Confirm that the peak area of the main peak obtained with 200 μL of this solution is equivalent to 2.0 to 3.0% of that obtained with 200 μL of the sample solution.

System performance: Dissolve 15 mg of egg albumin for gel filtration molecular mass marker and 15 mg of ribonuclease A for gel filtration molecular mass marker in 100 mL of tris-glycine buffer solution (pH 6.8). When the procedure is run with 20 μL of this solution under the above conditions, egg albumin and ribonuclease A are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 200 μL of the sample solution under the above operating conditions, the relative standard deviation of the area of the main peak is not more than 2.0%.

Bacterial endotoxins <*4.01*> Less than 0.25 EU per 600,000 Units.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To each well of a flat-bottom microplate add 45,000 to 60,000 cells of FL cell, prepared with Eagle's minimum essential medium containing bovine serum, and incubate at 37 ± 1°C for 18 to 22 hours in an incubator filled with 5%

carbon dioxide. Dilute Interferon Alfa (NAMALWA) Injection and Interferon Alfa RS separately with Eagle's minimum essential medium containing bovine serum so that each mL contains about 30 Units, and use these solutions as the sample solution (1) and the standard solution (1), respectively. To 200 μL each of these solutions add 117 μL of Eagle's minimum essential medium containing bovine serum, and use these solutions as the sample solution (2) and the standard solution (2), respectively. Repeat this operation, and prepare the sample solutions and standard solutions with logarithm dilutions of 8 serials (dilution ratio per stage is 0.2 $\log_{10}$ fold). Repeat to prepare the sample solutions three or more times. Add each sample solution or each standard solution into each well of the cell culture, and incubate at 37 ± 1°C for 6 hours. Discard the culture medium, add 1×10^5 to 1×10^6 PFU of Sindbis virus per well, and incubate at 37 ± 1°C for 38 to 42 hours. Discard the culture medium, add neutral red-Eagle's minimum essential medium containing bovine serum, and incubate at 37 ± 1°C for 45 to 75 minutes. Discard the culture medium, and add 0.01 mol/L phosphate buffer solution. Discard the liquid. Repeat this operation. Elute the neutral red that is taken up by the cells by adding sodium dihydrogen phosphate-ethanol TS. Determine the absorbance at 540 nm, prepare the dose-response curves by plotting the absorbances on the vertical axis and the logarithm of the dilution ratio on the horizontal axis with the absorbances obtained from the sample solution and standard solution. On the dose-response curves of the sample solution and standard solution, calculate the relative potency of the sample solution (n = 3 or more), prepared independently, to the standard solution by comparing the points where the intermediate of absorbances in cells infected with virus and cells not infected with virus, and calculate the average value of them as the potency of Interferon Alfa (NAMALWA) in 1 mL.

When all of the following conditions are satisfied, the test is valid.

Absorbance obtained from cells not infected with virus is 0.8 to 1.2.

Absorbance obtained from the cells infected with virus is not more than 0.1.

Standard deviation of the (log) potency of Interferon Alfa (NAMALWA) Injection in 1 mL obtained from the sample solution prepared three or more times independently is not more than 0.06.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, and at a temperature not exceeding 10°C, avoiding freezing.

Iodinated (^{131}I) Human Serum Albumin Injection

ヨウ化人血清アルブミン (^{131}I) 注射液

Iodinated (^{131}I) Human Serum Albumin Injection is an aqueous injection containing normal human serum albumin iodinated by iodine-131 (^{131}I).

It conforms to the requirements of Iodinated (^{131}I) Human Serum Albumin Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Iodinated (^{131}I) Human Serum Albumin Injection is a clear, colorless or light yellow liquid.

Iodine

ヨウ素

I: 126.90

Iodine contains not less than 99.5% of iodine (I).

Description Iodine occurs as grayish black, plates or granular, heavy crystals, having a metallic luster and a characteristic odor.

It is freely soluble in diethyl ether, soluble in ethanol (95), sparingly soluble in chloroform, and very slightly soluble in water.

It dissolves in potassium iodide TS.

It sublimes at room temperature.

Identification **(1)** A solution of Iodine in ethanol (95) (1 in 50) shows a red-brown color.

(2) A solution of Iodine in chloroform (1 in 1000) shows a red-purple to purple color.

(3) Add 0.5 mL of starch TS to 10 mL of a saturated solution of Iodine: a dark blue color is produced. When the mixture is boiled, the color disappears, and it reappears on cooling.

Purity **(1)** Non-volatile residue—Sublime 2.0 g of Iodine on a water bath, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

(2) Chloride or bromide—Mix 1.0 g of finely powdered Iodine with 20 mL of water, and filter the mixture. To 10 mL of the filtrate add dropwise diluted sulfurous acid solution (1 in 5) until the yellow color disappears. Add 1 mL of ammonia TS, followed by 1 mL of silver nitrate TS in small portions, and add water to make 20 mL. Shake well, filter, and after discarding the first 2 mL of the filtrate, take 10 mL of the subsequent filtrate. To the filtrate add 2.0 mL of nitric acid and water to make 20 mL: the solution so obtained has no more turbidity than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of water, 2.5 mL of ammonia TS, 1 mL of silver nitrate TS, 2.0 mL of nitric acid and water to make 20 mL.

Assay Place 1 g of potassium iodide and 1 mL of water in a glass-stoppered flask, weigh accurately, add about 0.3 g of Iodine to the flask, and weigh accurately again. Dissolve the iodine by gentle shaking, add 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 12.69 mg of I

Containers and storage Containers—Tight containers.

Iodine Tincture

ヨードチンキ

Iodine Tincture contains not less than 5.7 w/v% and not more than 6.3 w/v% of iodine (I: 126.90), and not less than 3.8 w/v% and not more than 4.2 w/v% of potassium iodide (KI: 166.00).

Method of preparation

Iodine	60 g
Potassium Iodide	40 g
70 vol% Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Spirits, with the above ingredients. It may be prepared with an appropriate quantity of Ethanol or Ethanol for Disinfection and Purified Water or Purified Water in Containers in place of 70 vol% Ethanol.

Description Iodine Tincture is a dark red-brown liquid, and has a characteristic odor.

Specific gravity d_{20}^{20}: about 0.97

Identification **(1)** To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Iodine Tincture: a dark blue-purple color develops.

(2) Evaporate 3 mL of Iodine Tincture to dryness on a water bath, and heat gently over a free flame: a white residue is formed which responds to the Qualitative Tests <*1.09*> for potassium salt and iodide.

Alcohol number <*1.01*> Not less than 6.6 (Method 2). Perform the pretreatment (ii) in the Method 1.

Assay **(1)** Iodine—Pipet 5 mL of Iodine Tincture, add 0.5 g of potassium iodide, 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 12.69 mg of I

(2) Potassium iodide—Pipet 5 mL of Iodine Tincture into an iodine flask, add 20 mL of water, 50 mL of hydrochloric acid and 5 mL of chloroform. Cool to room temperature, and titrate <*2.50*> with 0.05 mol/L potassium iodate VS until the red-purple color disappears from the chloroform layer, with agitating the mixture vigorously and continuously. After the chloroform layer has been decolorized, allow the mixture to stand for 5 minutes. If the color reappears, the mixture should be titrated <*2.50*> further with 0.05 mol/L potassium iodate VS. Calculate the amount (mg) of potassium iodide from the number of mL (a) of 0.05 mol/L potassium iodate VS used as above and the number of mL (b) of 0.1 mol/L sodium thiosulfate VS used in the titration under the Assay (1).

Amount (mg) of potassium iodide (KI)
$= 16.60 \times \{a - (b/2)\}$

Containers and storage Containers—Tight containers.

Dilute Iodine Tincture

希ヨードチンキ

Dilute Iodine Tincture contains not less than 2.8 w/v% and not more than 3.2 w/v% of iodine (I: 126.90), and not less than 1.9 w/v% and not more than 2.1 w/v% of potassium iodide (KI: 166.00).

Method of preparation

Iodine	30 g
Potassium Iodide	20 g
70 vol% Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Spirits, with the above ingredients. It may be prepared with an appropriate quantity of Ethanol or Ethanol for Disinfection and Purified Water or Purified Water in Containers in place of 70 vol% Ethanol. It may also be prepared by adding 70 vol% Ethanol to 500 mL of Iodine Tincture to make 1000 mL.

Description Dilute Iodine Tincture is a dark red-brown liquid, and has a characteristic odor.

Specific gravity d_{20}^{20}: about 0.93

Identification **(1)** To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Dilute Iodine Tincture: a dark blue-purple color develops.

(2) Evaporate 3 mL of Diluted Iodine Tincture to dryness on a water bath, and heat gently over a free flame: a white residue is formed which responds to the Qualitative Tests <*1.09*> for potassium salt and iodide.

Alcohol number <*1.01*> Not less than 6.7 (Method 2). Perform the pretreatment (ii) in the Method 1.

Assay **(1)** Iodine—Pipet exactly 10 mL of Dilute Iodine Tincture, add 0.5 g of potassium iodide, 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 12.69 mg of I

(2) Potassium iodide—Pipet 10 mL of Dilute Iodine Tincture into an iodine flask, add 20 mL of water, 50 mL of hydrochloric acid and 5 mL of chloroform. Cool to room temperature, and titrate <*2.50*> with 0.05 mol/L potassium iodate VS until the red-purple color in the chloroform layer disappears while agitating vigorously and continuously. After the chloroform layer has been decolorized, allow the mixture to stand for 5 minutes. If the color reappears, the mixture should be titrated <*2.50*> further with 0.05 mol/L potassium iodate VS. Calculate the amount (mg) of potassium iodide from the volume (a mL) of 0.05 mol/L potassium iodate VS consumed as above and the volume (b mL) of 0.1 mol/L sodium thiosulfate VS consumed in the titration under Assay (1).

Amount (mg) of potassium iodide (KI)
$= 16.60 \times \{a - (b/2)\}$

Containers and storage Containers—Tight containers.

Compound Iodine Glycerin

複方ヨード·グリセリン

Compound Iodine Glycerin contains not less than 1.1 w/v% and not more than 1.3 w/v% of iodine (I: 126.90), not less than 2.2 w/v% and not more than 2.6 w/v% of potassium iodide (KI: 166.00), not less than 2.7 w/v% and not more than 3.3 w/v% of total iodine (as I), and not less than 0.43 w/v% and not more than 0.53 w/v% of phenol (C_6H_6O: 94.11).

Method of preparation

Iodine	12 g
Potassium Iodide	24 g
Glycerin	900 mL
Mentha Water	45 mL
Liquefied Phenol	5 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Dissolve Potassium Iodide and Iodine in about 25 mL of Purified Water or Purified Water in Containers. After adding Glycerin, add Mentha Water, Liquefied Phenol and sufficient Purified Water or Purified Water in Containers to make 1000 mL, mixing thoroughly. It may be prepared with an appropriate quantity of Concentrated Glycerin and Purified Water or Purified Water in Containers in place of Glycerin, and with an appropriate quantity of Phenol and Purified Water or Purified Water in Containers in place of Liquefied Phenol.

Description Compound Iodine Glycerin is a red-brown, viscous liquid. It has a characteristic odor.

Specific gravity d_{20}^{20}: about 1.23

Identification **(1)** The colored solution obtained in the Assay (1) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 510 nm and 514 nm (iodine).

(2) The colored solution obtained in the Assay (2) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 510 nm and 514 nm (potassium iodide).

(3) The colored solution obtained in the Assay (4) has a yellow color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 401 nm and 405 nm (phenol).

(4) Take 1 mL of Compound Iodine Glycerin in a glass-stoppered test tube, add 10 mL of ethanol (95), and mix. Then add 2 mL of sodium hydroxide TS, add 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color develops (glycerin).

Assay **(1)** Iodine—Measure the specific gravity of Compound Iodine Glycerin according to Method 2 under Determination of Specific gravity and density <*2.56*>. Weigh exactly about 7 mL of it, add ethanol (95) to make exactly 200 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 80 mg of iodine for assay and about 0.17 g of potassium iodide for assay, previously dried at 105°C for 4 hours, dissolve in ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Pipet 3 mL each of the sample solution and the standard solution into 50-mL separators, to each add exactly 10 mL of a mixture of chloroform and hexane (2:1) and 15 mL of water successively, and shake immediately and vigorously. Separate the chloroform-hexane layers [use the water layers in (2)], and filter through a pledget of cotton. Determine the absorbances of the filtrates, A_T and A_S, at 512 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of iodine (I)} = M_S \times A_T/A_S$$

M_S: Amount (mg) of iodine for assay taken

(2) Potassium iodide—Separate the water layers of the sample solution and the standard solution obtained in (1), pipet 10 mL of each of the water layers, and to each add 1 mL of diluted dilute hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS and exactly 10 mL of a mixture of chloroform and hexane (2:1). Shake immediately and vigorously, separate the chloroform-hexane layers, and filter through a pledget of cotton. Determine the absorbances, A_T and A_S, of both solutions at 512 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of potassium iodide (KI)} = M_S \times A_T/A_S$$

M_S: Amount (mg) of potassium iodide for assay taken

(3) Total iodine—Measure the specific gravity of Compound Iodine Glycerin according to Method 2 under Determination of Specific gravity and density <*2.56*>. Weigh exactly about 5 mL of it, and add water to make exactly 50 mL. Pipet 5 mL of this solution into a 50-mL flask, and add 0.5 g of zinc powder and 5 mL of acetic acid (100). Shake until the color of iodine disappears, and heat under a reflux condenser on a water bath for 30 minutes. Wash the condenser with 10 mL of hot water, and filter through a glass filter (G3). Wash the flask with two 10-mL portions of warm water, and combine the filtrate and the washings. After cooling, add water to make exactly 50 mL, and use this solution as the sample solution. On the other hand, dissolve about 0.2 g of potassium iodide for assay, previously dried at 105°C for 4 hours and accurately weighed, in water to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of acetic acid (100) and water to make exactly 50 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution into 30-mL separators, and to each add 5 mL of water, 1 mL of diluted dilute hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS and 10 mL of a mixture of chloroform and hexane (2:1). Shake well immediately, and proceed as directed in (2).

$$\text{Amount (mg) of total iodine (I)} = M_S \times A_T/A_S \times 0.764$$

M_S: Amount (mg) of potassium iodide for assay taken

(4) Phenol—Measure the specific gravity of Compound Iodine Glycerin according to Method 2 under Determination of Specific gravity and density <*2.56*>. Weigh exactly about 2 mL of it, add 3 mL of 0.1 mol/L sodium thiosulfate VS, and shake. Add 2 mL of dilute hydrochloric acid, and shake with two 10-mL portions of chloroform. Separate the chloroform layer, and shake with two 10-mL portions of 0.5 mol/L sodium hydroxide TS. Separate the water layer, add water to make exactly 500 mL, and use this solution as the sample solution. Dissolve about 0.5 g of phenol for assay, accurately weighed, in ethanol (95) to make exactly 100 mL, pipet 2 mL of this solution, proceed in the same manner as the sample

solution, and use so obtaind solution as the standard solution. Pipet 3 mL each of the sample solution and standard solution, to each add 2 mL of dilute hydrochloric acid, and place in a water bath at 30°C. Allow to stand for 10 minutes, and add exactly 2 mL of a solution of sodium nitrite (1 in 100), shake, and allow to stand at 30°C for 60 minutes. Add dilute potassium hydroxide-ethanol TS to make exactly 25 mL, and determine the absorbances of these solutions, A_T and A_S, at 403 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution prepared in the same manner with 3 mL of water instead of the sample solution as the blank.

$$\text{Amount (mg) of phenol } (C_6H_6O) = M_S \times A_T/A_S \times 1/50$$

M_S: Amount (mg) of phenol for assay taken

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Dental Iodine Glycerin

歯科用ヨード·グリセリン

Dental Iodine Glycerin contains not less than 9.0 w/v% and not more than 11.0 w/v% of iodine (I: 126.90), not less than 7.2 w/v% and not more than 8.8 w/v% of potassium iodide (KI: 166.00), and not less than 0.9 w/v% and not more than 1.1 w/v% of zinc sulfate hydrate ($ZnSO_4.7H_2O$: 287.55).

Method of preparation

Iodine	10 g
Potassium Iodide	8 g
Zinc Sulfate Hydrate	1 g
Glycerin	35 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 100 mL

Dissolve and mix the above ingredients.

Description Dental Iodine Glycerin is a dark red-brown liquid, having the odor of iodine.

Identification **(1)** The colored solution obtained in the Assay (1) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 510 nm and 514 nm (iodine).

(2) The colored solution obtained in the Assay (2) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 510 nm and 514 nm (potassium iodide).

(3) Put 1 mL of Dental Iodine Glycerin in a glass-stoppered, test tube, add 10 mL of ethanol (95), and mix. Then add 2 mL of sodium hydroxide TS, add 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color develops (glycerin).

(4) The colored solution obtained in the Assay (3) acquires a red-purple to purple color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 618 nm and 622 nm (zinc sulfate hydrate).

Assay **(1)** Iodine—Pipet 5 mL of Dental Iodine Glycerin, and add diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 0.5 g of iodine for assay and about 0.4 g of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add exactly 20 mL of a mixture of chloroform and hexane (2:1), shake immediately, and separate the chloroform-hexane layer [use the water layer in (2)]. Filter through a pledget of cotton. Determine the absorbances, A_T and A_S, of the filtrates obtained from the sample solution and standard solution, respectively, at 512 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of iodine (I)} = M_S \times A_T/A_S$$

M_S: Amount (mg) of iodine for assay taken

(2) Potassium iodide—Separate the water layers of the sample solution and standard solution obtained in (1), pipet 7 mL each of the water layers, and to each add exactly 1 mL of diluted hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS and 10 mL of a mixture of chloroform and hexane (2:1), and shake immediately. Separate the chloroform-hexane layer, and filter through a pledget of cotton. Determine the absorbances, A_T and A_S, of the filtrates obtained from the sample solution and standard solution, respectively, at 512 nm as directed under Ultraviolet-visible Spectrophotometry, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of potassium iodide (KI)} = M_S \times A_T/A_S$$

M_S: Amount (mg) of potassium iodide for assay taken

(3) Zinc sulfate hydrate—Pipet 5 mL of Dental Iodine Glycerin, and add diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. On the other hand, pipet 10 mL of Standard Zinc Stock Solution, add diluted ethanol (3 in 200) to make exactly 1000 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add 10 mL of a mixture of chloroform and hexane (2:1), shake, and allow to stand. Pipet 3 mL each of the water layers, and to each add 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 10.0), 2 mL of zincon TS and water to make exactly 25 mL. Determine the absorbances, A_T and A_S, obtained from the sample solution and standard solution, respectively, at 620 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution prepared in the same manner with 3 mL of water as the blank.

$$\text{Amount (mg) of zinc sulfate hydrate } (ZnSO_4.7H_2O) = M_S \times A_T/A_S \times 4.398$$

M_S: Amount (mg) of zinc in 10 mL of Standard Zinc Stock Solution

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Iodine, Salicylic Acid and Phenol Spirit

ヨード·サリチル酸·フェノール精

Iodine, Salicylic Acid and Phenol Spirit contains not less than 1.08 w/v% and not more than 1.32 w/v% of iodine (I: 126.90), not less than 0.72 w/v% and not more than 0.88 w/v% of potassium iodide (KI: 166.00), not less than 4.5 w/v% and not more than 5.5 w/v% of salicylic acid ($C_7H_6O_3$: 138.12), not less than 1.8 w/v% and not more than 2.2 w/v% of phenol (C_6H_6O: 94.11), and not less than 7.2 w/v% and not more than 8.8 w/v% of benzoic acid ($C_7H_6O_2$: 122.12).

Method of preparation

Iodine Tincture	200 mL
Salicylic Acid	50 g
Phenol	20 g
Benzoic Acid	80 g
Ethanol for Disinfection	a sufficient quantity
	To make 1000 mL

Prepare as directed under Spirits, with the above ingredients. It may be prepared with an appropiate quantity of Ethanol and Purified Water or Purified Water in Containers in place of Ethanol for Disinfection.

Description Iodine, Salicylic Acid and Phenol Spirit is a dark red-brown liquid, having the odor of phenol.

Identification **(1)** To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Iodine, Salicylic Acid and Phenol Spirit: a dark blue-purple color develops (iodine).

(2) To 1 mL of Iodine, Salicylic Acid and Phenol Spirit add 5 mL of ethanol (95) and water to make 50 mL. To 1 mL of this solution add hydrochloric acid-potassium chloride buffer solution (pH 2.0) to make 50 mL, and to 15 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 25 mL of diethyl ether. Wash the diethyl ether extract with two 25-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is developed (phenol).

(4) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, extract with 10 mL of diethyl ether, and use the diethyl ether extract as the sample solution. Dissolve 25 mg of salicylic acid, 10 mg of phenol and 40 mg of benzoic acid in 5 mL each of diethyl ether, respectively, and use these solutions as the standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the 3 spots from the sample solution show the same Rf value as the corresponding spots of the standard solutions (1), (2) and (3). Spray evenly iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution acquires a purple color.

Assay **(1)** Iodine—Pipet 4 mL of Iodine, Salicylic Acid and Phenol Spirit, add ethanol (95) to make exactly 50 ml, and use this solution as the sample solution. On the other hand, weigh accurately about 1.2 g of iodine for assay and about 0.8 g of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 ml. Pipet 4 ml of this solution, add ethanol (95) to make exactly 50 ml, and use this solution as the standard solution. Pipet 3 ml each of the sample solution and standard solution, to each add exactly 25 ml of a mixture of chloroform and hexane (2:1), and shake. Further add exactly 10 ml of water, shake and separate the chloroform-hexane layers [use the water layers in (2)]. Filter through a pledget of absorbent cotton, and determine the absorbances of the filtrates from the sample solution and standard solution, respectively, A_T and A_S, at 512 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of iodine (I)} = M_S \times A_T/A_S \times 1/25$$

M_S: Amount (mg) of iodine for assay taken

(2) Potassium iodide—Separate the water layers of the sample solution and standard solution obtained in the Assay (1), pipet 8 ml each of the water layers, and add 1 ml of diluted dilute hydrochloric acid (1 in 2) and 1 ml of sodium nitrite TS. Immediately after shaking, add exactly 10 ml of a mixture of chloroform and hexane (2:1), shake, and proceed in the same manner as for the Assay (1).

$$\text{Amount (mg) of potassium iodide (KI)} = M_S \times A_T/A_S \times 1/25$$

M_S: Amount (mg) of potassium iodide for assay taken

(3) Salicylic acid, phenol and benzoic acid—Pipet 2 mL of Iodine, Salicylic Acid and Phenol Spirit, add 20 mL of diluted methanol (1 in 2) and 0.1 mol/L soium thiosulfate VS until the color of iodine disappears, add exactly 20 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 200 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, about 80 mg of phenol for assay, and 0.32 g of benzoic acid, previously dried in a desiccator (silica gel) for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 25 mL of this solution, add exactly 20 mL of the internal standard solution and diluted methanol (1 in 2) to make 200 mL, and use this solution as the standard solution. Perform the test with 3 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratios, Q_{Ta}, Q_{Tb} and Q_{Tc}, of the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the sample solution, and the ratios, Q_{Sa}, Q_{Sb} and Q_{Sc}, of the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the standard solution.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/2$$

$$\text{Amount (mg) of phenol } (C_6H_6O) = M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/2$$

Amount (mg) of benzoic acid ($C_7H_6O_2$)
$= M_{Sc} \times Q_{Tc}/Q_{Sc} \times 1/2$

M_{Sa}: Amount (mg) of salicylic acid for assay taken
M_{Sb}: Amount (mg) of phenol for assay taken
M_{Sc}: Amount (mg) of benzoic acid taken

Internal standard solution—A solution of theophylline in methanol (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 270 nm).
Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: Room temperature.
Mobile phase: A mixture of 0.1 mol/L phosohate buffer solution (pH 7.0) and methanol (3:1).
Flow rate: Adjust so that the retention time of salicylic acid is about 6 minutes.
Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 50 mg of theophylline in 100 mL of diluted ethanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 μL of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Iodoform

ヨードホルム

CHI_3: 393.73
Triiodomethane
[*75-47-8*]

Iodoform, when dried, contains not less than 99.0% of iodoform (CHI_3).

Description Iodoform occurs as lustrous, yellow crystals or crystalline powder. It has a characteristic odor.
It is freely soluble in diethyl ether, sparingly soluble in ethanol (95), and practically insoluble in water.
It is slightly volatile at ordinary temperature.
Melting point: about 120°C (with decomposition).

Identification Heat 0.1 g of Iodoform: a purple gas is evolved.

Purity **(1)** Water-soluble colored substances and acidity or alkalinity—Shake well 2.0 g of Iodoform, previously powdered, with 5 mL of water for 1 minute, allow to stand, and filter the supernatant liquid: the filtrate is colorless and neutral.
(2) Chloride <*1.03*>—Shake well 3.0 g of Iodoform, previously powdered, with 75 mL of water for 1 minute, allow to stand, and filter the supernatant liquid. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).
(3) Sulfate <*1.14*>—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

Loss on drying <*2.41*> Not more than 0.5% (1 g, silica gel, 24 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

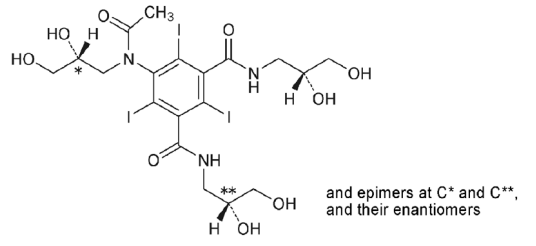
Assay Weigh accurately about 0.2 g of Iodoform, previously dried, in a 500-mL glass-stoppered flask, and dissolve it in 20 mL of ethanol (95). Add exactly 30 mL of 0.1 mol/L silver nitrate VS and 10 mL of nitric acid, stopper the flask, shake well, and allow to stand in a dark place over 16 hours. Add 150 mL of water, and titrate <*2.50*> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 5 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
$= 13.12$ mg of CHI_3

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Iohexol

イオヘキソール



$C_{19}H_{26}I_3N_3O_9$: 821.14
5-{Acetyl[(2*RS*)-2,3-dihydroxypropyl]amino}-*N*,*N*′-bis[(2*RS*)-2,3-dihydroxypropyl]-2,4,6-triiodobenzene-1,3-dicarboxamide
5-{Acetyl[(2*RS*)-2,3-dihydroxypropyl]amino}-*N*-[(2*RS*)-2,3-dihydroxypropyl]-*N*′-[(2*SR*)-2,3-dihydroxypropyl]-2,4,6-triiodobenzene-1,3-dicarboxamide
5-{Acetyl[(2*RS*)-2,3-dihydroxypropyl]amino}-*N*,*N*′-bis[(2*SR*)-2,3-dihydroxypropyl]-2,4,6-triiodobenzene-1,3-dicarboxamide
[*66108-95-0*]

Iohexol is a mixture of endo- and exo-products of iohexol.
It contains not less than 98.5% and not more than 101.0% of iohexol ($C_{19}H_{26}I_3N_3O_9$), calculated on the anhydrous basis.

Description Iohexol occurs as a white powder.
It is very soluble in water, freely soluble in methanol and sparingly soluble in ethanol (99.5).
It dissolves in a solution of sodium hydroxide (1 in 20).
A solution of Iohexol (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Iohexol (13 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Iohexol, previously dried at 105°C for 6 hours, as directed in the potassium bromide disk method under Infrared Spectro-

photometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Iohexol in 10 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (50:25:11) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of principal spots obtained from the sample solutions is two, and their *R*f values are about 0.2 and about 0.3, respectively.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Iohexol in 5 mL of water is clear and colorless.

(2) Aromatic primary amine—Conduct this procedure using light-resistant vessels. Dissolve 0.20 g of Iohexol in 15 mL of water, cool in ice for 5 minutes, add 1.5 mL of 6 mol/L hydrochloric acid TS and 1 mL of a solution of sodium nitrite (1 in 50), prepared before use, stir, and cool in ice for 4 minutes. Add 1 mL of a solution of amidosulfuric acid (standard reagent) (1 in 25), stir, and cool in ice for 1 minute. Then, add 0.5 mL of a solution, prepared by dissolving 0.3 g of *N*-1-naphthylethylenediamine dihydrochloride in diluted propylene glycol (7 in 10) to make 100 mL, and add water to make exactly 25 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> within 20 minutes, using a solution prepared in the same manner with 15 mL of water as the blank: the absorbance at 495 nm is not more than 0.21.

(3) Chloride <1.03>—Perform the test with 2.0 g of Iohexol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(4) Iodine and iodide—Dissolve 1.0 g of Iohexol in 4 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 10 minutes while occasional shaking. Add 5 mL of chloroform, shake well, and allow to stand: the chloroform layer is colorless. Then, add 1 mL of sodium nitrite solution (1 in 50), prepared before use, shake, allow to stand, and determine the absorbance of collected chloroform layer as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a chloroform layer prepared in the same manner with 4.0 mL of water as the blank: the absorbance at 510 nm is not larger than that of chloroform layer obtained from the following control solution.

Control solution: Dissolve exactly 0.131 g of potassium iodide in water to make exactly 100 mL. Pipet 1 mL of this solution, and add water to make exactly 100 mL. Pipet 3 mL of this solution, add 1 mL of water and 1 mL of dilute sulfuric acid, then proceed in the same manner.

(5) Heavy metals <1.07>—Proceed with 2.0 g of Iohexol according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) 3-Chloro-1,2-propanediol—To exactly 1.0 g of Iohexol, add exactly 2 mL of diethyl ether, and treat with ultrasonic waves for 10 minutes under cooling. Centrifuge, and use the diethyl ether layer as the sample solution. Separately, dissolve exactly 0.50 g of 3-chloro-1,2-propanediol in diethyl ether to make exactly 50 mL. Pipet 1 mL of this solution, and add diethyl ether to make exactly 100 mL. Pipet 5 mL of this solution, add diethyl ether to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02>, and determine the peak areas, A_T and A_S, of 3-chloro-1,2-propanediol in each solution: A_T is not larger than 2.5 times A_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface with a layer about 0.25 μm thick of 5% diphenyl-95% dimethylpolysiloxane for gas chromatography.

Column temperature: A constant temperature of about 70°C.

Injection port and detector temperature: A constant temperature of about 230°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of 3-chloro-1,2-propanediol is about 7 minutes.

Split ratio: 1:40.

System suitability—

System performance: To 1 mL of a solution of 3-chloro-1,2-propanediol in diethyl ether (1 in 200) and 1 mL of a solution of 1-hexanol in diethyl ether (1 in 800) add diethyl ether to make 200 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, 1-hexanol and 3-chloro-1,2-propanediol are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 3-chloro-1,2-propanediol is not more than 15%.

(7) Related substance—(i) Dissolve 1.0 g of Iohexol in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 25 mL. Pipet 1 mL of this solution, add the methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, 2-propanol, ammonia solution (28) and methanol (10:7:4:4) to a distance about 14 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot, other than the principal spot from the sample solution, appears at the relative *R*f value of 1.4 to the spot from the standard solution, is not more intense than the spot from the standard solution.

(ii) Dissolve 0.15 g of Iohexol in water to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area by the automatic integration method, and calculate the amounts by the area percentage method: the total amount of *O*-alkyl substances, having the relative retention time between 1.2 and 1.5 to the second principal peak (having bigger retention time) among the two principal peaks of iohexol, is not more than 0.6%, the amount of the peaks, which are eluted after the peak of iohexol and other than *O*-alkyl substances, is not more than 0.1%, respectively, and the total amount of the peaks, which are eluted after iohexol and other than *O*-alkyl substances, is not more than 0.3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diame-

ter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Acetonitrile.

Mobile phase B: Water.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 1	1	99
1 – 46	1 → 10	99 → 90

Flow rate: Adjust so that the retention time of the second principal peak (iohexol exo-product) is about 19 minutes.

Time span of measurement: About 2 times as long as the retention time of iohexol exo-product.

System suitability—

Test for required detectability: To 1 mL of the sample solution add water to make 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 20 mL. Confirm that the peak area of iohexol exo-product obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the resolution between the adjacent two peaks, which appear at the retention time of about 18 minutes, is not less than 1.5.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of iohexol exo-product is not more than 3.0%.

Water ⟨*2.48*⟩ Not more than 4.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Iohexol, dissolve in 25 mL of a solution of sodium hydroxide (1 in 20), add 0.5 g of zinc powder, boil under a reflux condenser for 30 minutes, and filter after cooling. Wash the flask and filter paper with 200 mL of water, combine the washings and filter, add 5 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L silver nitrate VS (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS) until the color of the precipitate changes from yellow to green.

Each mL of 0.1 mol/L silver nitrate VS
= 27.37 mg of $C_{19}H_{26}I_3N_3O_9$

Containers and storage Containers—Tight containers.

Iohexol Injection

イオヘキソール注射液

Iohexol Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iohexol ($C_{19}H_{26}I_3N_3O_9$: 821.14).

Method of preparation Prepare as directed under Injections, with Iohexol.

Description Iohexol Injection is a clear and colorless liquid.

Identification To a volume of Iohexol Injection, equivalent to 0.65 g of Iohexol, add water to make 500 mL. To 1 mL of this solution add water to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 243 nm and 247 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Purity (1) Aromatic primary amine—Conduct this procedure using light-resistant vessels. To a volume of Iohexol Injection, equivalent to 0.20 g of Iohexol add 15 mL of water, cool in ice for 5 minutes, add 1.5 mL of 6 mol/L hydrochloric acid TS and 1 mL of solution of sodium nitrite (1 in 50), prepared before use, shake, and cool in ice for 4 minutes. Then, proceed as directed in the Purity (2) under Iohexol: the absorbance of a solution so obtained is not more than 0.23.

(2) Iodine and iodide—To a volume of Iohexol Injection, equivalent to 1.0 g of Iohexol, add 4 mL of water and 1 mL of dilute sulfuric acid, and allow to stand for 10 minutes while occasional shaking. Then, proceed as directed in the Purity (4) under Iohexol: the absorbance of a chloroform layer so obtained is not more than 0.14.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.47 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

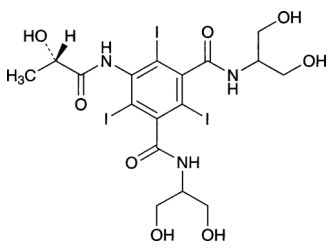
Assay To an exactly measured volume of Iohexol Injection, equivalent to about 1.5 g of iohexol ($C_{19}H_{26}I_3N_3O_9$), add water to make exactly 25 mL. Pipet 10 mL of this solution, add 25 mL of a solution of sodium hydroxide (1 in 20) and 0.5 g of zinc powder, and boil under a reflux condenser for 30 minutes. After cooling, wash down the inside of the condenser with 20 mL of water, and filter. Then, proceed as directed in the Assay under Iohexol.

Each mL of 0.1 mol/L silver nitrate VS
= 27.37 mg of $C_{19}H_{26}I_3N_3O_9$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Iopamidol

イオパミドール



$C_{17}H_{22}I_3N_3O_8$: 777.09

N,*N*′-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[(2*S*)-2-hydroxypropanoylamino]-2,4,6-triiodoisophthalamide [*62883-00-5*]

Iopamidol, when dried, contains not less than 99.0% of iopamidol ($C_{17}H_{22}I_3N_3O_8$).

Description Iopamidol occurs as a white crystalline powder.

It is very soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5).

Identification **(1)** To 50 mg of Iopamidol add 5 mL of hydrochloric acid, heat for 10 minutes in a water bath: the test solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(2) Heat 0.1 g of Iopamidol over a flame: a purple gas is evolved.

(3) Determine the infrared absorption spectrum of Iopamidol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_{436}^{20}$: −4.6 – −5.2° (after drying, 4 g, water, warm, after cooling, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Iopamidol in 10 mL of water: the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.60 g of Iopamidol in 8 mL of water, add 1 mL of a solution of sodium nitrite (1 in 50) and 12 mL of 2 mol/L hydrochloric acid TS, shake, and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate (1 in 10), shake well, allow to stand for 1 minute, and add 1 mL of naphthylethylenediamine TS and water to make exactly 50 mL. Determine the absorbance of this solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.12 (not more than 0.020%).

(3) Iodine—Dissolve 2.0 g of Iopamidol in 25 mL of water, add 5 mL of 1 mol/L sulfuric acid TS and 5 mL of toluene, shake well, and allow to stand: the toluene layer is colorless.

(4) Free iodine ion—Weigh accurately about 5 g of Iopamidol, dissolve in 70 mL of water, and adjust the pH to about 4.5 with dilute acetic acid. To this solution add 2 mL of 0.1 mol/L sodium chloride TS, and titrate <*2.50*> with 0.001 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.001 mol/L silver nitrate VS
= 0.1269 mg of I

Content of iodine ion in Iopamidol is not more than 0.001%.

(5) Heavy metals <*1.07*>—Moisten 1.0 g of Iopamidol with a small quantity of sulfuric acid, heat gradually to almost incinerate by a possibly lower temperature. After cooling, moisten again with a small quantity of sulfuric acid, heat gradually until white fumes no longer are evolved, and incinerate by ignition between 450 to 550°C. Proceed as directed in Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.10 g of Iopamidol in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area of the both solutions by the automatic integration method: each area of the peaks other than the peak of iopamidol from the sample solution is not larger than the peak area of the standard solution, and the total of these areas is not larger than 2.5 times of the peak area of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Water.

Mobile phase B: A mixture of water and methanol (3:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 6	92	8
6 – 18	92 → 65	8 → 35
18 – 30	65 → 8	35 → 92
30 – 34	8	92

Flow rate: 1.5 mL per minute.

Time span of measurement: About 4.3 times as long as the retention time of iopamidol.

System suitability—

System performance: Dissolve 1 mL of the sample solution and 10 mg of *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide in water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide and iopamidol are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the

peak areas of *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Iopamidol, previously dried, transfer to a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, boil for 30 minutes under a reflux confenser, cool, and filter. Wash the flask and the filter paper with 50 mL of water, and combine the washing with the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate ⟨*2.50*⟩ with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L sliver nitrate VS
= 25.90 mg of $C_{17}H_{22}I_3N_3O_8$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Iopamidol Injection

イオパミドール注射液

Iopamidol Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iopamidol ($C_{17}H_{22}I_3N_3O_8$: 777.09).

Method of preparation Prepare as directed under Injections, with Iopamidol.

Description Iopamidol Injection occurs as a clear, colorless or faint yellow, liquid, having slight viscosity.

It is gradually colored to faint yellow by light.

Identification **(1)** To a volume of Iopamidol Injection, equivalent to 0.3 g of Iopamidol, add 0.2 mL of sulfuric acid, and mix. When heat the solution over a flame, the color of the solution changes from colorless to purplish brown, and a purple gas is evolved.

(2) To a volume of Iopamidol Injection, equivalent to 0.6 g of Iopamidol, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 60 mg of iopamidol for assay in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 4 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, 2-butanone and ammonia solution (28) (2:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultra-violet light (main wavelength: 254 nm): the *R*f value of the principal spot obtained from the sample solution is the same as that obtained from the standard solution.

pH Being specified separately when the drug is granted approval based on the Law.

Purity **(1)** Primary aromatic amines—To a volume of Iopamidol Injection, equivalent to 0.18 g of Iopamidol, add 6 mL of water and mix. Add 1 mL of a solution of sodium nitrite (1 in 50) and 12 mL of 2 mol/L hydrochloric acid TS, shake the solution and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate (1 in 10), shake well, and allow to stand for 1 minute. Add 1 mL of naphthylethylenediamine TS and water to make exactly 50 mL. Determine the absorbance of this solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a solution prepared in the same manner as the blank: the absorbance is not more than 0.18.

(2) Iodine—Take a volume of Iopamidol Injection, equivalent to 2.0 g of Iopamidol, and add 2 mL of 1 mol/L sulfuric acid TS and 1 mL of toluene. Then shake well and allow to stand: the toluene layer is colorless.

(3) Free iodine ion—To exactly 10 mL of Iopamidol Injection add a suitable amount of water, and adjust the pH to about 4.5 with diluted 0.25 mol/L sulfuric acid TS (1 in 10). Titrate ⟨*2.50*⟩ with 0.001 mol/L silver nitrate VS (potentiometric titration): the amount of iodine ion contained in Iopamidol Injection is not more than 40 μg per mL.

Each mL of 0.001 mol/L silver nitrate VS = 0.1269 mg of I

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.5 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To exactly 1 mL of Iopamidol Injection add water to make exactly 200 mL. Take exactly *V* mL of this solution, add water to make exactly *V*′ mL so that each mL contains about 80 μg of iopamidol ($C_{17}H_{22}I_3N_3O_8$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of iopamidol for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 10 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of iopamidol in each solution.

Amount (mg) of iopamidol ($C_{17}H_{22}I_3N_3O_8$)
$= M_S \times A_T/A_S \times V'/V \times 4/5$

M_S: Amount (mg) of iopamidol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Water.

Mobile phase B: A mixture of water and methanol (3:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 6	92	8
6 – 18	92 → 65	8 → 35
18 – 30	65 → 8	35 → 92
30 – 34	8	92

Flow rate: 1.5 mL per minute.

System suitability—

System performance: Dissolve 1 mg each of iopamidol for assay and *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide in water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide and iopamidol are eluted in this order with the resolution between these peaks being not less than 7.

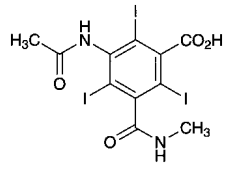
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of iopamidol is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Light-resistant.

Iotalamic Acid

イオタラム酸



$C_{11}H_9I_3N_2O_4$: 613.91

3-Acetylamino-2,4,6-triiodo-5-(methylaminocarbonyl)benzoic acid

[*2276-90-6*]

Iotalamic Acid, when dried, contains not less than 99.0% of iotalamic acid ($C_{11}H_9I_3N_2O_4$).

Description Iotalamic Acid occurs as a white powder. It is odorless.

It is sparingly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

It gradually colored by light.

Identification **(1)** Heat 0.1 g of Iotalamic Acid over a flame: a purple gas is evolved.

(2) Determine the infrared spectrum of Iotalamic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Iotalamic Acid in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Primary aromatic amines—To 0.50 g of Iotalamic Acid add 15 mL of water, and dissolve it in 1 mL of sodium hydroxide TS while ice-cooling. Add 4 mL of a solution of sodium nitrite (1 in 100) to the solution, immediately add 12 mL of 1 mol/L hydrochloric acid TS, and shake gently. Then allow the mixture to stand for exactly 2 minutes, add 8 mL of ammonium amidosulfate TS, and shake occasionally for 5 minutes. Add 3 drops of a solution of 1-naphthol in ethanol (95) (1 in 10), allow to stand for 1 minute, add 3.5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), mix, and immediately add water to make 50 mL. Determine within 20 minutes the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.25.

(3) Soluble halides—Dissolve 0.5 g of Iotalamic Acid in 20 mL of diluted ammonia TS (1 in 40), add 6 mL of dilute nitric acid, shake, allow to stand for 5 minutes, and filter. Transfer the filtrate to a Nessler tube, wash the residue with 20 mL of water, combine the filtrate and the washings, and add water to make 50 mL. Proceed as directed for the Chloride Limit Test <*1.03*> using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS and add 20 mL of diluted ammonia TS (1 in 40), 6 mL of dilute nitric acid and water to make 50 mL.

(4) Iodine—Dissolve 0.20 g of Iotalamic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, and allow to stand for 10 minutes with occasional shaking. Add 5 mL of chloroform, shake well, and allow to stand: the chloroform layer remains colorless.

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of Iotalamic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <*1.11*>—Prepare the test solution with 0.6 g of Iotalamic Acid according to Method 3, and perform the test (not more than 3.3 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Iotalamic Acid, previously dried, place it in a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, and heat for 30 minutes under a reflux condenser. Cool, filter, wash the flask and the filter paper with 50 mL of water, and combine the washings and the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS, until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

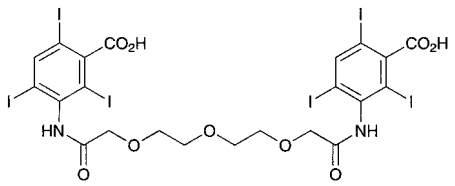
Each mL of 0.1 mol/L silver nitrate VS
= 20.46 mg of $C_{11}H_9I_3N_2O_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Iotroxic Acid

イオトロクス酸



$C_{22}H_{18}I_6N_2O_9$: 1215.81
3,3′-(3,6,9-Trioxaundecanedioyl)diiminobis-(2,4,6-triiodobenzoic acid)
[*51022-74-3*]

Iotroxic Acid contains not less than 98.5% of iotroxic acid ($C_{22}H_{18}I_6N_2O_9$), calculated on the anhydrous basis.

Description Iotroxic Acid occurs as a white crystalline powder.

It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is gradually colored by light.

Identification **(1)** Heat 0.1 g of Iotroxic Acid over a flame: a purple gas evolves.

(2) Dissolve a suitable amount of Iotroxic Acid in a suitable amount of methanol, evaporate the methanol under reduced pressure, and determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Iotroxic Acid in 10 mL of diluted sodium hydroxide TS (1 in 5): the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Iotroxic Acid in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, mix, and allow to stand for 2 minutes. Add 5 mL of ammonium amidosulfate TS, shake well, allow to stand for 1 minute, then add 0.4 mL of a solution of α-naphthol in ethanol (95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL. Read the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a blank solution obtained in the same manner as above: the absorbance is not more than 0.22.

(3) Iodine—Dissolve 0.20 g of Iotroxic Acid in 2.0 mL of sodium hydrogen carbonate TS, add 5 mL of toluene, mix well, and allow to stand: the toluene layer is colorless.

(4) Free iodine ion—Weigh accurately about 5.0 g of Iotroxic Acid, dissolve in 12 mL of a solution of meglumine (3 in 20), add water to make 70 mL, and adjust the pH to about 4.5 with acetic acid (100). To this solution add 2 mL of 0.1 mol/L sodium chloride TS, and titrate <*2.50*> with 0.001 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.001 mol/L silver nitrate
= 0.1269 mg of I

Content of iodine ion in Iotroxic Acid, calculated on the anhydrous basis, is not more than 0.004%.

(5) Heavy metals <*1.07*>—Heat strongly 1.0 g of Iotroxic Acid as directed under Residue on Ignition Test <*2.44*>, then proceed according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.15 g of Iotroxic Acid in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, acetone and formic acid (6:4:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 1.0 – 2.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Iotroxic Acid, dissolve in 40 mL of sodium hydroxide TS in a saponification flask, add 1 g of zinc powder, and boil for 30 minutes under a reflux condenser. After cooling, filter, wash the flask and the filter paper with 50 mL of water, and combine the washings to the filtrate. To this solution add 5 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 20.26 mg of $C_{22}H_{18}I_6N_2O_9$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ipratropium Bromide Hydrate

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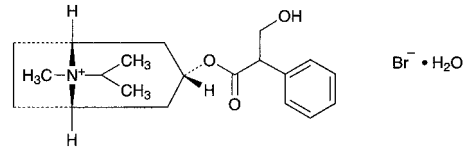


$C_{20}H_{30}BrNO_3.H_2O$: 430.38
(1*R*,3*r*,5*S*)-3-[(2*RS*)-3-Hydroxy-2-phenylpropanoyloxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane bromide monohydrate
[*66985-17-9*]

Ipratropium Bromide Hydrate, when dried, contains not less than 99.0% of ipratropium bromide ($C_{20}H_{30}BrNO_3$: 412.36).

Description Ipratropium Bromide Hydrate occurs as a white crystalline powder.

It is freely soluble in water, soluble in ethanol (99.5), slightly soluble in acetonitrile and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Ipratropium Bromide Hydrate in 20 mL of water is between 5.0 and 7.5.

Melting point: about 223°C (with decomposition, after drying).

Identification **(1)** To 5 mg of Ipratropium Bromide Hydrate add 0.5 mL of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 5 mL of acetone, and add 2 drops of potassium hydroxide-ethanol TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of Ipratropium Bromide Hydrate in 0.01 mol/L hydrochloric acid TS (3 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ipratropium Bromide Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) The solution of Ipratropium Bromide Hydrate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for bromide.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ipratropium Bromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate ⟨*1.14*⟩—Perform the test with 1.0 g of Ipratropium Bromide Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ipratropium Bromide Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Ipratropium Bromide Hydrate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 1 ppm).

(5) Isopropylatropine bromide—Dissolve 25 mg of Ipratropium Bromide Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Perform the test with 25 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak area, A_a, of ipratropium and the peak area, A_b, having the relative retention time to ipratropium about 1.3 by the automatic integration method: $A_b/(A_a + A_b)$ is not more than 0.01, and no peak other than the peak of ipratropium and the peak having the relative retention time to ipratropium about 1.3 appears within about 14 minutes of the retention time after the solvent peak.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and 10 to 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200), acetonitrile and methanesulfonic acid (1000:120:1).

Flow rate: Adjust so that the retention time of ipratropium is about 7 minutes.

Selection of column: Heat a solution of Ipratropium Bromide Hydrate in 1 mol/L hydrochloric acid TS (1 in 100) at 100°C for 1 hour, and cool. To 2.5 mL of this solution add the mobile phase to make 100 mL. Proceed with 25 μL of this solution under the above operating conditions, and calculate the resolution. Use a column showing a resolution not less than 3 between the peak of ipratropium and the peak having the relative retention time to ipratropium about 0.6.

Detection sensitivity: Adjust so that the peak height of ipratropium obtained from 25 μL of the sample solution composes 50 to 80% of the full scale.

(6) Apo-compounds—Dissolve 0.14 g of Ipratropium Bromide Hydrate in 0.01 mol/L hydrochloric acid TS to make 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_1 and A_2, at 246 nm and 263 nm, respectively: A_1/A_2 is not more than 0.91.

Loss on drying ⟨*2.41*⟩ 3.9 – 4.4% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

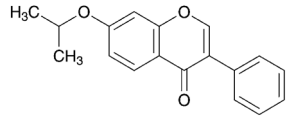
Assay Weigh accurately about 0.3 g of Ipratropium Bromide Hydrate, previously dried, dissolve in 40 mL of acetic acid (100), add 40 mL of 1,4-dioxane and 2.5 mL of bismuth nitrate TS, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.24 mg of $C_{20}H_{30}BrNO_3$

Containers and storage Containers—Tight containers.

Ipriflavone

イプリフラボン



$C_{18}H_{16}O_3$: 280.32
7-(1-Methylethyl)oxy-3-phenyl-4*H*-chromen-4-one
[*35212-22-7*]

Ipriflavone, when dried, contains not less than 98.5% and not more than 101.0% of ipriflavone ($C_{18}H_{16}O_3$).

Description Ipriflavone occurs as white to yellowish white, crystals or crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It gradually turns yellow on exposure to light.

Identification **(1)** Determine the absorption spectrum of a solution of Ipriflavone in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ipriflavone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ipriflavone as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Ipriflavone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 116 – 119°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ipriflavone according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Ipriflavone according to Method 4, and perform the test. Prepare the test solution with 10 mL of dilute hydrochloric acid instead of using 3 mL of hydrochloric acid. Prepare the standard color with 1.0 mL of Standard Arsenic Solution (not more than 1 ppm).

(3) Related substances—Dissolve 30 mg of Ipriflavone in 50 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of ipriflavone obtained from the sample solution is not larger than 1/2 times the peak area of ipriflavone obtained from the standard solution, and the total area of the peaks other than the peak of ipriflavone from the sample solution is not larger than the peak area of ipriflavone from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of ipriflavone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of ipriflavone obtained from 20 μL of this solution is equivalent to 7 to 13% of that of ipriflavone obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ipriflavone are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ipriflavone to that of the internal standard is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Ipriflavone and Ipriflavone RS, previously dried, dissolve separately in acetonitrile to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and acetonitrile to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ipriflavone to that of the internal standard.

$$\text{Amount (mg) of ipriflavone } (C_{18}H_{16}O_3) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Ipriflavone RS taken

Internal standard solution—A solution of di-*n*-butyl phthalate in acetonitrile (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and water (3:2).

Flow rate: Adjust so that the retention time of ipriflavone is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ipriflavone and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ipriflavone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ipriflavone Tablets

イプリフラボン錠

Ipriflavone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ipriflavone ($C_{18}H_{16}O_3$: 280.32).

Method of preparation Prepare as directed under Tablets, with Ipriflavone.

Identification To a quantity of powdered Ipriflavone Tablets, equivalent to 11 mg of Ipriflavone, add 100 mL of methanol, shake vigorously for 10 minutes, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 247 nm and 251 nm, and between 297 nm and 301 nm.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately the mass of not less than 20 Ipriflavone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 30 mg of ipriflavone ($C_{18}H_{16}O_3$), add 30 mL of acetonitrile, shake vigorously for 15 minutes, add acetonitrile to make exactly 50 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and add acetonitrile to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Ipriflavone RS, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Ipriflavone.

Amount (mg) of ipriflavone ($C_{18}H_{16}O_3$)
$= M_S \times Q_T/Q_S$

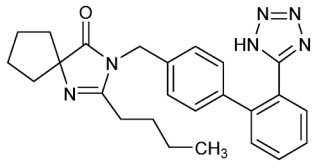
M_S: Amount (mg) of Ipriflavone RS taken

Internal standard solution—A solution of di-*n*-butyl phthalate in acetonitrile (1 in 100).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Irbesartan

イルベサルタン



$C_{25}H_{28}N_6O$: 428.53
2-Butyl-3-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1,3-diazaspiro[4.4]non-1-en-4-one
[*138402-11-6*]

Irbesartan contains not less than 99.0% and not more than 101.0% of irbesartan ($C_{25}H_{28}N_6O$), calculated on the anhydrous basis.

Description Irbesartan occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5) and practically insoluble in water.

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Irbesartan in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Irbesartan as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the Irbesartan in methanol, evaporate the solvent, dry the residue, and perform the test using the residue.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Irbesartan according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Irbesartan in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 0.8 to irbesartan, obtained from the sample solution is not larger than 1.5 times the peak area of irbesartan from the standard solution, the area of the peak other than irbesartan and the peak mentioned above from the sample solution is not larger than the peak area of irbesartan from the standard solution, and the total area of the peaks other than irbesartan from the sample solutions is not larger than 2 times the peak area of irbesartan from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 5.5 mL of phosphoric acid add 950 mL of water, and adjust to pH 3.2 with triethylamine. To 670 mL of this solution add 330 mL of acetonitrile for liquid chromatography.

Flow rate: 1.0 mL per minute.

Time span of measurement: About 1.4 times as long as the retention time of irbesartan, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of irbesartan obtained with 10 μL of this solution is equivalent to 35 to 65% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of irbesartan are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of irbesartan is not more than 3.0%.

(3) Azides—Being specified separately when the drug is granted approval based on the Law.

Water <*2.48*> Not more than 0.5% (1 g, volumetric titration, back titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

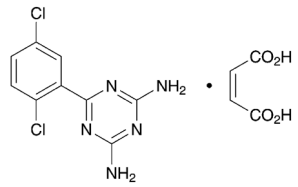
Assay Weigh accurately about 0.3 g of Irbesartan, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
$= 42.85$ mg of $C_{25}H_{28}N_6O$

Containers and storage Containers—Tight containers.

Irsogladine Maleate

イルソグラジンマレイン酸塩



$C_9H_7Cl_2N_5.C_4H_4O_4$: 372.16
6-(2,5-Dichlorophenyl)-1,3,5-triazine-2,4-diamine monomaleate
[*84504-69-8*]

Irsogladine Maleate, when dried, contains not less than 99.0% and not more than 101.0% of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$).

Description Irsogladine Maleate occurs as white, crystals or crystalline powder. It has a slightly bitter taste.

It is sparingly soluble in acetic acid (100) and in ethyleneglycol, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Dissolve 20 mg of Irsogladine Maleate in methanol to make 20 mL. Take 2 mL of this solution, and add water to make 20 mL. To 2 mL of this solution add water to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Irsogladine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 10 mg of Irsogladine Maleate in 1 mL of dilute hydrochloric acid and 4 mL of water, and add 3 drops of potassium permanganate TS: the color of the solution is discharged immediately.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Irsogladine Maleate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Irsogladine Maleate in 10 mL of ethylene glycol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethylene glycol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of maleic acid and irsogladine obtained from the sample solution is not larger than 1/10 times the peak area of irsogladine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanesulfonic acid solution (1 in 1000) and methanol (4:1).

Flow rate: Adjust so that the retention time of irsogladine is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of irsogladine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add ethylene glycol to make exactly 10 mL. Confirm that the peak area of irsogladine obtained from 5 μL of this solution is equivalent to 7 to 13% of that of irsogladine obtained from 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of irsogladine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of irsogladine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Irsogladine Maleate, previously dried, dissolve in 25 mL of acetic acid (100), add 25 mL of acetic anhydride, and titrate <*2.50*> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 18.61 mg of $C_9H_7Cl_2N_5.C_4H_4O_4$

Containers and storage Containers—Well-closed containers.

Irsogladine Maleate Fine Granules

イルソグラジンマレイン酸塩細粒

Irsogladine Maleate Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$: 372.16).

Method of preparation Prepare as directed under Granules, with Irsogladine Maleate.

Identification To a quantity of powdered Irsogladine Maleate Fine Granules, equivalent to 2 mg of Irsogladine Maleate, add 5 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of irsogladine maleate in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of petroleum ether, acetone and acetic acid (100) (12:4:1) to a distance of about 10 cm, and air-

dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same *R*f value as the spot from the standard solution.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Irsogladine Maleate Fine Granules in single-dose packages meet the requirement of the Content uniformity test.

Take out the total contents of 1 package of Irsogladine Maleate Fine Granules, add 2 mL of water, add 2 mL methanol per mg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$), treat with ultrasonic waves for 10 minutes with occasional shaking, and add water to make exactly V mL so that each mL contains about 40 μg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$). Centrifuge this solution, pipet 1 mL of the supernatant liquid, and add water to make exactly 20 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$)
$= M_S \times A_T/A_S \times V/500$

M_S: Amount (mg) of irsogladine maleate for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Irsogladine Maleate Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Irsogladine Maleate Fine Granules, equivalent to about 4 mg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Dissolution rate (%) with respect to the labeled amount of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 9$

M_S: Amount (mg) of irsogladine maleate for assay taken
M_T: Amount (g) of Irsogladine Maleate Fine Granules taken
C: Labeled amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$) in 1 g

Assay Weigh accurately an amount of powdered Irsogladine Maleate Fine Granules, equivalent to about 5 mg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$), add exactly 5 mL of the internal standard solution, shake until it is dispersed, and add 5 mL of water. To the solution add 25 mL of ethylene glycol, treat with ultrasonic waves for 10 minutes with occasional shaking, and add ethylene glycol to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, and dissolve in ethylene glycol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 5 mL of water and ethylene glycol to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of irsogladine to that of the internal standard.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of irsogladine maleate for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (750:250:3).

Flow rate: Adjust so that the retention time of irsogladine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, irsogladine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of irsogladine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Irsogladine Maleate Tablets

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Irsogladine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$: 372.16).

Method of preparation Prepare as directed under Tablets, with Irsogladine Maleate.

Identification To a quantity of powdered Irsogladine Maleate Tablets, equivalent 2 mg of Irsogladine Maleate, add 5 mL of methanol, shake for 10 minutes, centrifuge, and use

the supernatant liquid as the sample solution. Separately, dissolve 2 mg of irsogladine maleate in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of petroleum ether, acetone and acetic acid (100) (12:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same *R*f value as the spot from the standard solution.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Irsogladine Maleate Tablets add 2 mL of water, add 2 mL of methanol per mg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$), treat with ultrasonic waves for 10 minutes with occasional shaking, add water to make exactly *V* mL so that each mL contains about 40 μg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$). Centrifuge this solution, pipet 1 mL of the supernatant liquid, and add water to make exactly 20 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$)
$= M_S \times A_T/A_S \times V/500$

M_S: Amount (mg) of irsogladine maleate for assay taken

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Irsogladine Maleate Tablets is not less than 80%.

Start the test with 1 tablet of Irsogladine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V′* mL so that each mL contains about 2.2 μg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Dissolution rate (%) with respect to the labeled amount of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of irsogladine maleate for assay taken
C: Labeled amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Irsogladine Maleate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$), add exactly 5 mL of the internal standard solution, shake until it is dispersed, and add 5 mL of water. To this solution add 25 mL of ethylene glycol, treat with ultrasonic waves for 10 minutes with occasional shaking, and add ethylene glycol to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of irsogladine maleate for assay, previously dried at 105°C for 4 hours, and dissolve in ethylene glycol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 5 mL of water and ethylene glycol to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of irsogladine to that of the internal standard.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5.C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of irsogladine maleate for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (750:250:3).

Flow rate: Adjust so that the retention time of irsogladine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, irsogladine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of irsogladine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Isepamicin Sulfate

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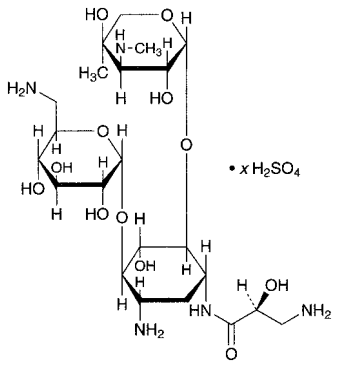

$C_{22}H_{43}N_5O_{12}.xH_2SO_4$

6-Amino-6-deoxy-α-D-glucopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-1-*N*-[(2*S*)-3-amino-2-hydroxypropanoyl]-D-streptamine sulfate

[*67814-76-0*]

Isepamicin Sulfate is the sulfate of a derivative of gentamycin B, an aminoglycoside substance, having antibacterial activity produced by the growth of *Micromonospora purpurea*.

It contains not less than 680 μg (potency) and not more than 780 μg (potency) per mg, calculated on the anhydrous basis. The potency of Isepamicin Sulfate is expressed as mass (potency) of isepamicin ($C_{22}H_{43}N_5O_{12}$: 569.60).

Description Isepamicin Sulfate occurs as a white to pale yellowish white powder.

It is very soluble in water, and practically insoluble in methanol and in ethanol (95).

It is hygroscopic.

Identification **(1)** Dissolve 20 mg of Isepamicun Sulfate in 1 mL of water, add 3 mL of anthrone TS, shake, and allow to stand: a blue-purple color develops.

(2) Dissolve 10 mg each of Isepamicin Sulfate and Isepamicin Sulfate RS in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia water (28), ethanol (99.5), 1-butanol and chloroform (5:5:4:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at about 100°C for about 10 minutes: the principal spots from the sample solution and the spot from the standard solution exhibit a red-brown color and show the same *R*f value.

(3) Dissolve 10 mg of Isepamicin Sulfate in 1 mL of water, and add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +100 – +120° (0.25 g calculated on the anhydrous bases, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.5 g of Isepamicin Sulfate in 5 mL of water: the pH of the solution is between 5.5 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Isepamicin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Isepamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate the amounts of their peaks by the area percentage method: the amount of HAPA-gentamine-B equivalent to about 0.4 of the relative retention time to isepamicin is not more than 5.0%, and gentamicin B equivalent to about 1.3 of that is not more than 3.0%. For the peak area of gentamicin B, multiply the relative response factor, 1.11.

Operating conditions—

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of mobile phase, and flow rate of reagent: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of isepamicin.

System suitability—

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To 1 mL of the sample solution, add water to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of isepamicin obtained from 5 μL of this solution is equivalent to 7 to 13% of that obtained from 5 μL of the solution for system suitability test.

Water <*2.48*> Not more than 12.0% (0.2 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Residue on ignition <*2.44*> Not more than 1.0% (1 g).

Assay Weigh accurately an amount of Isepamicin Sulfate and Isepamicin Sulfate RS, equivalent to about 20 mg (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of isepamicin in each solution.

Amount [μg (potency)] of isepamicin ($C_{22}H_{43}N_5O_{12}$) $= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Isepamicin Sulfate RS taken

Operating conditions—

Apparatus: Consist of two pumps for the mobile phase and the reagent transport, inject port, column, reaction coil, detector and recorder. Use a reaction coil with thermostat.

Detector: A fluorophotometer (excitation wavelength: 360 nm, detection wavelength: 440 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

25°C.

Reaction coil: A column 0.25 mm in inside diameter and 5 m in length.

Mobile phase: Dissolve 28.41 g of anhydrous sodium sulfate and 5.23 g of sodium 1-pentane sulfonate in 900 mL of water, add 1 mL of acetic acid (100), and add water to make exactly 1000 mL.

Reagent: To 500 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 10.0) add 5 mL of a solution of *o*-phthalaldehyde in ethanol (95) (2 in 25), 1 mL of 2-mercaptoethanol and 2 mL of a solution of lauromacrogol (1 in 4).

Reaction temperature: A constant temperature of about 45°C.

Flow rate of mobile phase: About 0.6 mL per minute.

Flow rate of reagent: About 0.5 mL per minute.

System suitability—

System performance: Dissolve 2 mg of gentamicin B in 10 mL of the standard solution. When the procedure is run with 5 μL of this solution under the above operating conditions, isepamicin and gentamicin B are eluted in this order with the resolution between these peaks being not less than 1.0.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of isepamicin is not more than 3.0%.

Containers and storage Containers—Tight containers.

Isepamicin Sulfate Injection

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Isepamicin Sulfate Injection is an aqueous injection. It contains not less than 90.0% and not more than 110.0% of the labeled potency of isepamicin ($C_{22}H_{43}N_5O_{12}$: 569.60).

Method of preparation Prepare as directed under Injections, with Isepamicin Sulfate.

Description Isepamicin Sulfate Injection is a clear, colorless liquid.

Identification To a volume of Isepamicin Sulfate Injection, equivalent to 20 mg (potency) of Isepamicin Sulfate, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve an amount of Isepamicin Sulfate RS, equivalent to 20 mg (potency) in 10 mL of water, and use this solution as the standard solution. Proceed with these solutions as directed in the Identification (2) under Isepamicin Sulfate.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH ⟨*2.54*⟩ 5.5 – 7.5.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of their peaks by the area percentage method: the amount of isoserine, having the relative retention time of about 0.3 to isepamicin, is not more than 2.0%, and the amount of gentamicin B, having the relative retention time of about 1.3 to isepamicin, is not more than 4.0%. For the peak area of gentamicin B, multiply the relative response factor, 1.11.

Operating conditions—

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reaction reagent, reaction temperature, flow rate of mobile phase, and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Assay under Isepamicin Sulfate.

Time span of measurement: About 2 times as long as the retention time of isepamicin.

System suitability—

System performance and system repeatability: Proceed as directed in the Assay under Isepamicin Sulfate.

Test for required detectability: To 1 mL of the sample solution add water to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of isepamicin obtained from 5 μL of this solution is equivalent to 7 to 13% of that of isepamicin obtained from 5 μL of the solution for system suitability test.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mg (potency).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Isepamicin Sulfate Injection, equivalent to about 0.2 g (potency) of Isepamicin Sulfate, add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Isepamicin Sulfate RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Isepamicin Sulfate.

Amount [mg (potency)] of isepamicin ($C_{22}H_{43}N_5O_{12}$) $= M_S \times A_T/A_S \times 10$

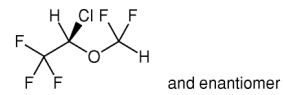
M_S: Amount [mg (potency)] of Isepamicin Sulfate RS taken

Containers and storage Containers—Hermetic containers.

Shelf life 24 months after preparation.

Isoflurane

イソフルラン



and enantiomer

$C_3H_2ClF_5O$: 184.49

(2*RS*)-2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane [*26675-46-7*]

Isoflurane contains not less than 99.0% and not more than 101.0% of isoflurane ($C_3H_2ClF_5O$), calculated on the anhydrous basis.

Description Isoflurane occurs as a clear, colorless fluid liquid.

It is miscible with ethanol (99.5), with methanol and with *o*-xylene.

It is slightly soluble in water.

It is volatile, and has no inflammability.

It shows no optical rotation.

Refractive index n_D^{20}: about 1.30

Boiling point: about 47 – 50°C

Identification **(1)** The test solution obtained by the Oxygen Flask Combustion Method ⟨*1.06*⟩ with 50 μL of Isoflurane, using 40 mL of water as the absorbing liquid, responds to the Qualitative Tests ⟨*1.09*⟩ for chloride and fluoride.

(2) Determine the infrared absorption spectrum of Isoflurane as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Isoflurane RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.500 – 1.520

Purity **(1)** Acidity or alkalinity—To 10 mL of Isoflurane add 5 mL of freshly boiled and cooled water, and shake for 1 minute: the water layer is neutral.

(2) Soluble chloride—To 60 g of Isoflurane add 40 mL of water, shake thoroughly, and separate the water layer. To 20 mL of the layer add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as directed under Chloride Limit Test ⟨*1.03*⟩. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 3 ppm).

(3) Soluble fluoride—To 6 g of Isoflurane add 12 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and shake for 10 minutes. Transfer 4.0 mL of the water layer into a Nessler tube, add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Separately, to 0.4 mL of the fluorine standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a Nessler tube add 30 mL of the mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1), then proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, obtained by proceeding in the same manner as above with 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), as the blank: the absorbance of the sample solution is not more than that of the standard solution (not more than 2 ppm).

Fluorine standard solution: Dissolve exactly 2.21 g of sodium fluoride in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of fluorine (F).

(4) Related substances—Use Isoflurane as the sample solution. To exactly 1 mL of the sample solution add *o*-xylene to make exactly 100 mL. Pipet 1 mL of this solution, add *o*-xylene to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than isoflurane from sample solution is not larger than the peak area of isoflurane from the standard solution, and the total area of the peaks other than isoflurane from the sample solution is not larger than 3 times the peak area of isoflurane from the standard solution.

Operating conditions—

Detector, column, column temperature, carrier gas, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of isoflurane.

System suitability—

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 1 mL of the standard solution add *o*-xylene to make exactly 2 mL. Confirm that the peak area of isoflurane obtained with 5 μL of this solution is equivalent to 35 to 65% of that obtained with 5 μL of the standard solution.

(5) Peroxide—Take 10 mL of Isoflurane in a Nessler tube add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake vigorously, and allow to stand in a dark place for 1 hour: the water layer is not yellow.

(6) Residue on evaporation—Pipet 65 mL of Isoflurane, evaporate on a water bath, and dry the residue at 105°C for 1 hour: not more than 1.0 mg.

Water ⟨*2.48*⟩ Not more than 0.1% (2 g, Coulometric titration).

Assay To exactly 5 mL each of Isoflurane and Isoflurane RS (separately determined the water ⟨*2.48*⟩ in the same manner as Isoflurane), add exactly 3 mL of ethyl acetate as the internal standard, then add *o*-xylene to make 50 mL each. To 5 mL each of these solutions add *o*-xylene to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of isoflurane to that of the internal standard.

Amount (mg) of isoflurane ($C_3H_2ClF_5O$) in 5 mL of Isoflurane

$$= V_S \times Q_T/Q_S \times 1000 \times 1.506$$

V_S: Amount (mL) of Isoflurane RS taken, calculated on the anhydrous basis

1.506: Specific gravity (d_{20}^{20}) of isoflurane

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column 3 mm in inside diameter and 3.5 m in length, packed with siliceous earth for gas chromatography (125 – 149 μm in particle diameter), coated in 10% with nonylphenoxypoly(ethyleneoxy)ethanol for gas chromatography and in 15% with polyalkylene glycol for gas chromatography.

Column temperature: A constant temperature of about 80°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of isoflurane is about 7 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, isoflurane and the internal standard are eluted in this

order with the resolution between these peaks being not less than 3.

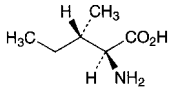
System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoflurane is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At a temperature not exceeding 30°C.

L-Isoleucine

L-イソロイシン



$C_6H_{13}NO_2$: 131.17
(2*S*,3*S*)-2-Amino-3-methylpentanoic acid
[*73-32-5*]

L-Isoleucine, when dried, contains not less than 98.5% of L-isoleucine ($C_6H_{13}NO_2$).

Description L-Isoleucine occurs as white, crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Isoleucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +39.5 – +41.5° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of L-Isoleucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Isoleucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Isoleucine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Isoleucine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Isoleucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Dissolve 1.0 g of L-Isoleucine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of L-Isoleucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Isoleucine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.13 g of L-Isoleucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.12 mg of $C_6H_{13}NO_2$

Containers and storage Containers—Tight containers.

L-Isoleucine, L-Leucine and L-Valine Granules

イソロイシン·ロイシン·バリン顆粒

L-Isoleucine, L-Leucine and L-Valine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of L-isoleucine ($C_6H_{13}NO_2$: 131.17), L-leucine ($C_6H_{13}NO_2$: 131.17) and L-valine ($C_5H_{11}NO_2$: 117.15).

Method of preparation Prepare as directed under Granules, with L-Isoleucine, L-Leucine and L-Valine.

Identification Dissolve an amount of powdered L-Isoleucine, L-Leucine and L-Valine Granules, equivalent to about 92 mg of L-Isoleucine, in the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, dissolve 0.46 g of L-isoleucine, 0.92 g of L-leucine and 0.55 g of L-valine in the mobile phase to make 100 mL. Take 10 mL of this solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention times of the peak in the chromatograms obtained from the sample solution and the standard solution are the same.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 31.2 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 2.8

with phosphoric acid. To 970 mL of this solution add 30 mL of acetonitrile.

Flow rate: Adjust so that the retention time of L-valine is about 2.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, valine, isoleucine and leucine are eluted in this order, and the resolution between the peaks of isoleucine and leucine is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the retention time of isoleucine, leucine and valine are not more than 1.0%, respectively.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: the Granules in single-dose package meets the requirement of the Content uniformity test.

To the total content of 1 package of L-Isoleucine, L-Leucine and L-Valine Granules add exactly $V/25$ mL of the internal standard solution, and add 0.1 mol/L hydrochloric acid TS to make V mL so that each mL contains about 3.8 mg of L-isoleucine ($C_6H_{13}NO_2$). To 2 mL of this solution add 0.02 mol/L hydrochloric acid TS to make 200 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of L-isoleucine ($C_6H_{13}NO_2$)
$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times V/50$
Amount (mg) of L-leucine ($C_6H_{13}NO_2$)
$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times V/50$
Amount (mg) of L-valine ($C_5H_{11}NO_2$)
$= M_{Sc} \times Q_{Tc}/Q_{Sc} \times V/50$

M_{Sa}: Amount (mg) of L-isoleucine for assay taken
M_{Sb}: Amount (mg) of L-leucine for assay taken
M_{Sc}: Amount (mg) of L-valine for assay taken

Internal standard solution—A solution of glycine in 0.1 mol/L hydrochloric acid TS (1 in 20).

Disintegration ⟨*6.09*⟩ It meets the requirement. Carry out the test for 15 minutes.

Assay Powder the total amount of the content of not less than ten packages of L-Isoleucine, L-Leucine and L-Valine Granules. Weigh accurately a portion of the powder, equivalent to about 0.95 g of L-isoleucine ($C_6H_{13}NO_2$), add exactly 10 mL of the internal standard solution, dissolve in 0.1 mol/L hydrochloric acid TS to make 250 mL. To 2 mL of this solution add 0.02 mol/L hydrochloric acid TS to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.2 g of L-isoleucine for assay, about 0.4 g of L-leucine for assay and about 0.24 g of L-valine for assay, previously these are dried at 105°C for 3 hours, add exactly 2 mL of the internal standard solution, dissolve in 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.02 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Calculate the ratios, Q_{Ta}, Q_{Tb} and Q_{Tc} of the peak area of L-isoleucine, L-leucine and L-valin to that of the internal standard obtained from the sample solution, and the ratios, Q_{Sa}, Q_{Sb} and Q_{Sc} of the peak area of L-isoleucine, L-leucine and L-valin to that of the internal standard from the standard solution.

Amount (mg) of L-isoleucine ($C_6H_{13}NO_2$)
$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 5$
Amount (mg) of L-leucine ($C_6H_{13}NO_2$)
$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 5$
Amount (mg) of L-valine ($C_5H_{11}NO_2$)
$= M_{Sc} \times Q_{Tc}/Q_{Sc} \times 5$

M_{Sa}: Amount (mg) of L-isoleucine for assay taken
M_{Sb}: Amount (mg) of L-leucine for assay taken
M_{Sc}: Amount (mg) of L-valine for assay taken

Internal standard solution—A solution of glycine in 0.1 mol/L hydrochloric acid TS (1 in 20).

Operating conditions—

Detector: A visible absorption photometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 6 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated polystyrene (3 μm in particle diameter) (sodium type).

Column temperature: A constant temperature of about 57°C.

Reaction vessel temperature: A constant temperature of about 130°C.

Reaction time: About 1 minute.

Mobile phase: After prepare the mobile phases A, B, C, D and E according to the following table, add 0.1 mL caprylic acid to each mobile phase.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	—
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	—
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	—
Sodium hydroxide	—	—	—	—	8.00 g
Ethanol (99.5)	130 mL	20 mL	4 mL	—	100 mL
Thiodiglycol	5 mL	5 mL	5 mL	—	—
Benzyl alcohol	—	—	—	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL	4 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Switching of mobile phase: Switch the mobile phases A, B, C, D and E sequentially so that when proceed with 20 μL of the standard solution under the conditions above, the internal standard, valine, isoleucine and leucine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagent: Dissolve 407 g of lithium acetate dihydrate in an appropriate amount of water, add 245 mL of acetic acid (100), 801 mL of 1-methoxy-2-propanol and water to make 2000 mL, pass nitrogen for 10 minutes, and use this solution as Solution (I). Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin, pass nitrogen for 5 minutes, add 0.161 g of sodium borohydride, and pass nitrogen for 30 minutes. To this solution add an equal volume of the Solution (I). Prepare before use.

Flow rate of mobile phase: 0.40 mL per minute.

Flow rate of reaction regent: 0.35 mL per minute.

System suitability—

System performance: When the test is run with 20 μL of

the standard solution under the above operating conditions, the internal standard, valine, isoleucine and leucine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

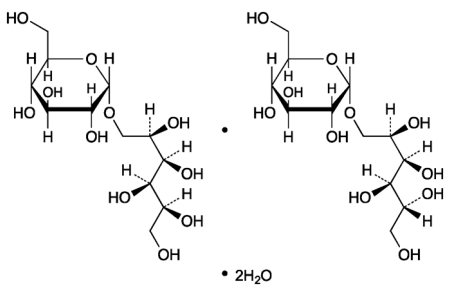
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of isoleucine, leucine and valine to that of the internal standard are not more than 1.0%, respectively.

Containers and storage Containers—Tight containers.

Isomalt Hydrate

Isomalt

イソマル水和物



6-*O*-α-D-Glucopyranosyl-D-glucitol $C_{12}H_{24}O_{11}$: 344.31
1-*O*-α-D-Glucopyranosyl-D-mannitol dihydrate $C_{12}H_{24}O_{11}.2H_2O$: 380.34
6-*O*-α-D-Glucopyranosyl-D-glucitol—1-*O*-α-D-glucopyranosyl-D-mannitol dihydrate
[*64519-82-0*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Isomalt Hydrate is a mixture of 6-*O*-α-D-glucopyranosyl-D-sorbitol and 1-*O*-α-D-glucopyranosyl-D-mannitol.

It contains not less than 98.0% and not more than 102.0% as the mixture of 6-*O*-α-D-glucopyranosyl-D-sorbitol ($C_{12}H_{24}O_{11}$) and 1-*O*-α-D-glucopyranosyl-D-mannitol ($C_{12}H_{24}O_{11}$), calculated on the anhydrous basis, and the amount of each component is not less than 3.0%, respectively.

The label states the contents (%) of 6-*O*-α-D-glucopyranosyl-D-sorbitol and 1-*O*-α-D-glucopyranosyl-D-mannitol.

◆**Description** Isomalt Hydrate occurs as a white, powder or grains.

It is freely soluble in water, and practically insoluble in ethanol (95).

Optical rotation $[\alpha]_D^{20}$: about + 92°(1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).◆

Identification ◆**(1)** To 1 mL of a solution of Isomalt Hydrate (1 in 100) add 1 mL of a solution of catechol (1 in 10) prepared before use, shake thoroughly, add 2 mL of sulfuric acid rapidly, and shake: a reddish purple to red-purple color develops.◆

(2) Dissolve 1.0 g of Isomalt Hydrate in water to make 50 mL, and use this solution as the sample solution. ◆Separately, dissolve 0.2 g of Isomalt RS in water to make 10 mL, and use this solution as the standard solution.◆ Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from these solutions: the two principal peaks, 6-*O*-α-D-glucopyranosyl-D-sorbitol and 1-*O*-α-D-glucopyranosyl-D-mannitol, in the chromatogram obtained from the sample solution are similar in retention time to respective two peaks obtained from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Purity ◆**(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Isomalt Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(2) Nickel—Weigh exactly an amount of Isomalt Hydrate, equivalent to 10.0 g calculated on the anhydrous basis, dissolve in 30 mL of 2 mol/L acetic acid TS, and add water to make exactly 100 mL. Add exactly 2 mL of a solution of ammonium pyrrolidinedithiocarbamate (1 in100) and exactly 10 mL of water-saturated 4-methyl-2-pentanone, and shake for 30 seconds protected from light. Allow the layers to separate, and use the 4-methyl-2-pentanone layer as the sample solution. Separately, take in three vessels three exact portions of Isomalt Hydrate, each equivalent to 10.0 g calculated on the anhydrous basis, add 30 mL of 2 mol/L acetic acid TS to them, then add exactly 0.5 mL, 1.0 mL and 1.5 mL respectively of Standard Nickel Solution for Atomic Absorption Spectrophotometry, and add water to make them exactly 100 mL. Then, proceed in the same manner as the sample solution, and use the solutions so obtained as the standard solutions. Separately, prepare 4-methyl-2-pentanone layer by proceeding in the same manner as the sample solution but omitting the substance to be examined, and use this as the blank. Perform the test with the sample solution and standard solution as directed in Standard addition method under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions. The blank is used to set the zero of the instrument, and to ascertain that the readings return to zero after rinsing the sample introduction system with water between each measurement: the amount of nickel is not more than 1 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Nickel hollow-cathode lamp.
Wavelength: 232.0 nm.

(3) Related substances—Weigh exactly 1.00 g of Isomalt Hydrate, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exacty 10.0 mg of D-sorbitol and 10.0 mg of D-mannitol, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of D-mannitol, having a relative retention time of about 1.6 to 1-*O*-α-D-glucopyranosyl-D-mannitol, and D-sorbitol, having a relative retention time of about 2.0, obtained from the sample solution are not larger than the area of the corresponding peak obtained from the standard solution (not more than 0.5%), and the area of the peak other than the peaks of 1-*O*-α-D-glucopyranosyl-D-mannitol and 6-*O*-α-D-glucopyranosyl-D-

sorbitol having a relative retention time of about 1.2 to 1-*O*-α-D-glucopyranosyl-D-mannitol and the peaks mentioned above from the sample solution is not larger than the peak area of D-sorbitol from the standard solution (not more than 0.5%). In addition, the total area of the peaks other than 1-*O*-α-D-glucopyranosyl-D-mannitol and 6-*O*-α-D-glucopyranosyl-D-sorbitol from the sample solution is not larger than 4 times the peak area of D-sorbitol from the standard solution (not more than 2.0%). However, the peaks which area is not larger than 1/5 times the peak area of D-sorbitol from the standard solution are disregarded (not more than 0.1%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of 1-*O*-α-D-glucopyranosyl-D-mannitol.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◆Test for required detectability: Pipet 2 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of D-sorbitol obtained with 20 μL of this solution is equivalent to 14 to 26% of that of D-sorbitol obtained with 20 μL the standard solution.◆

◆System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of D-mannitol and D-sorbitol are not more than 2.0%, respectively◆.

(4) Reducing sugars—Dissolve 3.3 g of Isomalt Hydrate in 10 mL of water with the aid of gentle heat, cool, and add 20 mL of copper (II) citrate TS. Add a few amount of boiling chips, heat so that the boiling begins after 4 minutes, and maintain boiling for 3 minutes. Cool rapidly, add 100 mL of a solution of acetic acid (100) (3 in 125) and exactly 20 mL of 0.025 mol/L iodine VS. With continuous shaking, add 25 mL of a mixture of water and hydrochloric acid (47:3). When the precipitate has dissolved, titrate ⟨*2.50*⟩ the excess of iodine with 0.05 mol/L sodium thiosulfate VS, until the blue color due to 1 mL of soluble starch TS added at near of the end point disappears: not less than 12.8 mL of 0.05 mol/L sodium thiosulfate VS is required (not more than 0.3% as glucose).

Conductivity ⟨*2.51*⟩ Dissolve 20 g of Isomalt Hydrate in a suitable amount of freshly boiled and cooled water with the aid of gentle heat at 40 – 50°C, cool, add the same water to make exactly 100 mL, and use this solution as the sample solution. Measure the conductivity (25°C) of the sample solution at 25 ± 0.1°C while gently stirring with a magnetic stirrer: not more than 20 $\mu S \cdot cm^{-1}$.

Water ⟨*2.48*⟩ Not more than 7.0% (0.3 g, volumetric titration, direct titration. Use a mixture of methanol for water determination and formamide for water determination (1:1) heated at 50 ± 5°C instead of methanol for water determination).

Assay Weight accurately about 1 g of Isomalt Hydrate, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. ◆Separately, weigh accurately about 0.2 g of Isomalt RS (separately determine the water ⟨*2.48*⟩ in the same manner as Isomalt Hydrate), dissolve in water to make exactly 10 mL, and use this solution as the standard solution.◆ Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_{Ta} and A_{Tb}, and A_{Sa} and A_{Sb}, of 1-*O*-α-D-glucopyranosyl-D-mannitol and 6-*O*-α-D-glucopyranosyl-D-sorbitol in each solution.

Amount (g) of 1-*O*-α-D-glucopyranosyl-D-mannitol ($C_{12}H_{24}O_{11}$)
$= M_S \times K_a/100 \times A_{Ta}/A_{Sa}$

Amount (g) of 6-*O*-α-D-glucopyranosyl-D-sorbitol ($C_{12}H_{24}O_{11}$)
$= M_S \times K_b/100 \times A_{Tb}/A_{Sb}$

M_S: Amount (g) of Isomalt RS taken, calculated on the anhydrous basis
K_a: Content (%) of 1-*O*-α-D-glucopyranosyl-D-mannitol ($C_{12}H_{24}O_{11}$) in Isomalt RS
K_b: Content (%) of 6-*O*-α-D-glucopyranosyl-D-sorbitol ($C_{12}H_{24}O_{11}$) in Isomalt RS

Operating conditions—

Detector: A differential refractometer maintained at a constant temperature (40°C for example).

Column: Two stainless steel columns, 4.6 mm in inside diameter and 3 cm in length, and 7.8 mm in inside diameter and 30 cm in length, both packed with strong acid ion-exchange resin (Ca type) for liquid chromatography with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with divinylbenzene (degree of cross-linkage: 8%) (9 μm in particle diameter). These are used as the pre-column and the separation column, respectively.

Column temperature: 80 ± 3°C.

Mobile phase: Water.

Flow rate: 0.5 mL per minute (retention time of 1-*O*-α-D-glucopyranosyl-D-mannitol is about 12 minutes).

System suitability—

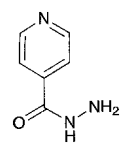
System performance: When the procedure is run with 20 μL of the standard solution under these above operating conditions, 1-*O*-α-D-glucopyranosyl-D-mannitol and 6-*O*-α-D-glucopyranosyl-D-sorbitol are eluted in this order with the resolution between these peaks being not less than 2.0.

◆System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-*O*-α-D-glucopyranosyl-D-mannitol and 6-*O*-α-D-glucopyranosyl-D-sorbitol is not more than 2.0%, respectively.◆

◆**Containers and storage** Containers—Well-closed containers.◆

Isoniazid

イソニアジド



$C_6H_7N_3O$: 137.14
Pyridine-4-carbohydrazide
[*54-85-3*]

Isoniazid, when dried, contains not less than 98.5% of isoniazid ($C_6H_7N_3O$).

Description Isoniazid occurs as colorless crystals or a white crystalline powder. It is odorless.

It is freely soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and very slightly soluble in diethyl ether.

Identification **(1)** Dissolve about 20 mg of Isoniazid in water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Isoniazid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Dissolve 1.0 g of Isoniazid in 10 mL of freshly boiled and cooled water: the pH of this solution is between 6.5 and 7.5.

Melting point <*2.60*> 170 – 173°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Isoniazid in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Isoniazid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Isoniazid according to Method 3, and perform the test. In this case, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite the ethanol to burn (not more than 5 ppm).

(4) Hydrazine—Dissolve 0.10 g of Isoniazid in 5 mL of water, add 0.1 mL of a solution of salicylaldehyde in ethanol (95) (1 in 20), shake immediately, and allow to stand for 5 minutes: no turbidity is produced.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Isoniazid, previously dried, dissolve in 50 mL of acetic acid (100) and 10 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.71 mg of $C_6H_7N_3O$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Isoniazid Injection

イソニアジド注射液

Isoniazid Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of isoniazid ($C_6H_7N_3O$: 137.14).

Method of preparation Prepare as directed under Injections, with Isoniazid.

Description Isoniazid Injection occurs as a clear, colorless liquid.

pH: 6.5 – 7.5

Identification To a volume of Isoniazid Injection, equivalent to 20 mg of Isoniazid, and add water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 264 nm and 268 nm.

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Isoniazid Injection, equivalent to about 50 mg of isoniazid ($C_6H_7N_3O$), add water to make exactly 100 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of isoniazid for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of isoniazid to that of the internal standard.

Amount (mg) of isoniazid ($C_6H_7N_3O$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of isoniazid for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL. Separately, to 5.76 g of phosphoric acid add water to make 1000 mL. Mix these solutions to make a solution having pH 2.5. To 500 mL of

this solution add 500 mL of methanol, and add 2.86 g of sodium tridecanesulfonate to dissolve.

Flow rate: Adjust so that the retention time of isoniazid is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, isoniazid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of isoniazid to that of the internal standard is not more than 1.3%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Isoniazid Tablets

イソニアジド錠

Isoniazid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of isoniazid ($C_6H_7N_3O$: 137.14).

Method of preparation Prepare as directed under Tablets, with Isoniazid.

Identification Take a quantity of powdered Isoniazid Tablets, equivalent to 20 mg of Isoniazid, add 200 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 264 nm and 268 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Isoniazid Tablets add water to make exactly V mL so that each mL contains about 0.5 mg of isoniazid ($C_6H_7N_3O$), and shake well to disintegrate. Filter this solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = M_S \times A_T/A_S \times V/100$$

M_S: Amount (mg) of isoniazid for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Isoniazid Tablets is not less than 75%.

Start the test with 1 tablet of Isoniazid Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of isoniazid for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL, then pipet 5 mL of this solution, add water to make exactly 50 mL, and then pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 267 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Dissolution rate (\%) with respect to the labeled amount of isoniazid } (C_6H_7N_3O) = M_S \times A_T/A_S \times 1/C \times 90$$

M_S: Amount (mg) of isoniazid for assay taken

C: Labeled amount (mg) of isoniazid ($C_6H_7N_3O$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Isoniazid Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.1 g of isoniazid ($C_6H_7N_3O$), add 150 mL of water, shake for 30 minutes, then add water to make exactly 200 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of isoniazid for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas, A_T and A_S, of isoniazid in each solution.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of isoniazid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL. Separately, to 5.76 g of phosphoric acid add water to make 1000 mL. Mix these solutions to adjust the pH to 2.5. To 400 mL of this solution add 600 mL of methanol, and add 2.86 g of sodium tridecanesulfonate to dissolve.

Flow rate: Adjust so that the retention time of isoniazid is about 5 minutes.

System suitability—

System performance: Dissolve 5 mg of Isoniazid and 5 mg of isonicotinic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, isonicotinic acid and isoniazid are eluted in this order with the resolution between these peaks being not less than 1.5.

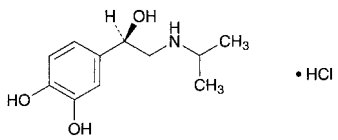
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoniazid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

l-Isoprenaline Hydrochloride

l-イソプレナリン塩酸塩



$C_{11}H_{17}NO_3.HCl$: 247.72
4-{(1*R*)-1-Hydroxy-
2-[(1-methylethyl)amino]ethyl}benzene-
1,2-diol monohydrochloride
[*5984-95-2*]

l-Isoprenaline Hydrochloride, when dried, contains not less than 98.0% of *l*-isoprenaline hydrochloride ($C_{11}H_{17}NO_3.HCl$).

Description *l*-Isoprenaline Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in acetic acid (100), in acetic anhydride, in diethyl ether and in chloroform.

It gradually changes in color by air and by light.

Identification **(1)** Dissolve 10 mg of *l*-Isoprenaline Hydrochloride in 5 mL of water, and add 1 drop of iron (III) chloride TS: a deep green color develops, and changes through yellow-green to brown on standing.

(2) Dissolve 1 mg each of *l*-Isoprenaline Hydrochloride in 1 mL of water in the test tubes A and B. Add 10 mL of potassium hydrogen phthalate buffer solution (pH 3.5) to A, and add 10 mL of phosphate buffer solution (pH 6.5) to B. To each of the test tubes add 1 mL of iodine TS, allow to stand for 5 minutes, and add 2 mL each of sodium thiosulfate TS: a red color develops in the test tube A, and a deep red color develops in the test tube B.

(3) Dissolve 10 mg of *l*-Isoprenaline Hydrochloride in 1 mL of water, and add 1 mL of phosphotungstic acid TS: a light brown precipitate is produced.

(4) Determine the absorption spectrum of a solution of *l*-Isoprenaline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) A solution of *l*-Isoprenaline Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -36 – $-41°$ (after drying, 0.25 g, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.10 g of *l*-Isoprenaline Hydrochloride in 10 mL of water: the pH of the solution is between 4.5 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of *l*-Isoprenaline Hydrochloride in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Perform the test with 0.10 g of *l*-Isoprenaline Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.192%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of *l*-Isoprenaline Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Isoproterenone—Dissolve 50 mg of *l*-Isoprenaline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: not more than 0.040.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of *l*-Isoprenaline Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and acetic anhydride (3:2) by warming, cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

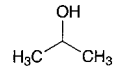
Each mL of 0.1 mol/L perchloric acid VS
= 24.77 mg of $C_{11}H_{17}NO_3.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Isopropanol

Isopropyl Alcohol

イソプロパノール



C_3H_8O: 60.10
Propan-2-ol
[*67-63-0*]

Description Isopropanol is a clear, colorless liquid. It has a characteristic odor.

It is miscible with water, with methanol, with ethanol (95), and with diethyl ether.

It is flammable and volatile.

Identification **(1)** To 1 mL of Isopropanol add 2 mL of iodine TS and 2 mL of sodium hydroxide TS, and shake: a light yellow precipitate is formed.

(2) To 5 mL of Isopropanol add 20 mL of potassium dichromate TS and 5 mL of sulfuric acid with caution, and warm gently on a water bath: the produced gas has the odor of acetone, and the gas turns the filter paper, previously wetted with a solution of salicylaldehyde in ethanol (95) (1 in 10) and with a solution of sodium hydroxide (3 in 10), to red-brown.

Specific gravity <*2.56*> d_{20}^{20}: 0.785 – 0.788

Purity **(1)** Clarity of solution—To 2.0 mL of Isopropanol add 8 mL of water, and shake: the solution is clear.

(2) Acidity—To 15.0 mL of Isopropanol add 50 mL of freshly boiled and cooled water and 2 drops of phenolphthalein TS, and add 0.40 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Residue on evaporation—Evaporate 20.0 mL of Isopropanol on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Water <*2.48*> Not more than 0.75 w/v% (2 mL, volumetric titration, direct titration).

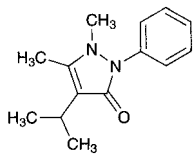
Distilling range <*2.57*> 81 – 83°C, not less than 94 vol%.

Containers and storage Containers—Tight containers.
Storage—Remote from fire.

Isopropylantipyrine

Propyphenazone

イソプロピルアンチピリン



$C_{14}H_{18}N_2O$: 230.31
1,5-Dimethyl-4-(1-methylethyl)-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one
[*479-92-5*]

Isopropylantipyrine, when dried, contains not less than 98.0% of isopropylantipyrine ($C_{14}H_{18}N_2O$).

Description Isopropylantipyrine occurs as white, crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in acetic acid (100), freely soluble in ethanol (95) and in acetone, soluble in diethyl ether, and slightly soluble in water.

Identification (1) To 2 mL of a solution of Isopropylantipyrine (1 in 500) add 1 drop of iron (III) chloride TS: a light red color develops. Further add 3 drops of sulfuric acid to this solution: the color changes to pale yellow.

(2) Add 5 mL of a solution of Isopropylantipyrine (1 in 500) to a mixture of 5 mL of potassium hexacyanoferrate (III) TS and 1 to 2 drops of iron (III) chloride TS: a dark green color gradually develops.

(3) To 2 mL of a solution of Isopropylantipyrine (1 in 500) add 2 to 3 drops of tannic acid TS: a white precipitate is produced.

Melting point ⟨*2.60*⟩ 103 – 105°C

Purity (1) Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute ethanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid, 30 mL of dilute ethanol and water to make 50 mL (not more than 0.014%).

(2) Sulfate ⟨*1.14*⟩—Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute ethanol, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and 30 mL of dilute ethanol, and dilute with water to make 50 mL (not more than 0.019%).

(3) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Isopropylantipyrine in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 25 mL of acetone, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Isopropylantipyrine according to Method 3, and perform the test (not more than 2 ppm).

(5) Antipyrine—Dissolve 1.0 g of Isopropylantipyrine in 10 mL of dilute ethanol, and add 1 mL of sodium nitrite TS and 1 mL of dilute sulfuric acid: no green color develops.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

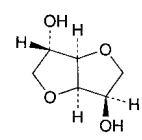
Assay Weigh accurately about 0.4 g of Isopropylantipyrine, previously dried, dissolve in 60 mL of a mixture of acetic acid (100) and acetic anhydride (2:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 23.03 mg of $C_{14}H_{18}N_2O$

Containers and storage Containers—Tight containers.

Isosorbide

イソソルビド



$C_6H_{10}O_4$: 146.14
1,4:3,6-Dianhydro-D-glucitol
[*652-67-5*]

Isosorbide contains not less than 98.5% of isosorbide ($C_6H_{10}O_4$), calculated on the anhydrous basis.

Description Isosorbide occurs as white, crystals or masses. It is odorless, or has a faint, characteristic odor, and has a bitter taste.

It is very soluble in water and in methanol, freely soluble in ethanol (95), and slightly soluble in diethyl ether.

It is hygroscopic.

Identification (1) To 0.1 g of Isosorbide add 6 mL of diluted sulfuric acid (1 in 2), and dissolve by heating in a water bath. After cooling, shake well with 1 mL of a solution of potassium permanganate (1 in 30), and heat in a water bath until the color of potassium permanganate disappears. To this solution add 10 mL of 2,4-dinitrophenylhydrazine TS, and heat in a water bath: an orange precipitate is formed.

(2) To 2 g of Isosorbide add 30 mL of pyridine and 4 mL of benzoyl chloride, boil under a reflux condenser for 50 minutes, cool, and pour gradually the solution into 100 mL of cold water. Filter the formed precipitate by suction through a glass filter (G3), wash with water, recrystallize twice from ethanol (95), and dry in a desiccator (in vacuum, silica gel) for 4 hours: it melts ⟨*2.60*⟩ between 102°C and 103°C.

(3) Determine the infrared absorption spectrum of Isosorbide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +45.0 – +46.0° (5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Take 25 g of

Isosorbide in a Nessler tube, and dissolve in 50 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add water to make 10.0 mL. To 3.0 mL of this solution add water to make 50 mL.

(2) Sulfate <*1.14*>—Perform the test with 2.0 g of Isosorbide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <*1.07*>—Proceed with 5.0 g of Isosorbide according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Isosorbide according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Isosorbide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of ethanol (95) and sulfuric acid (9:1) on the plate, and heat at 150°C for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 1.5% (2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

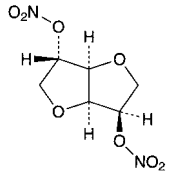
Assay Weigh accurately about 10 g of Isosorbide, calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL. Determine the optical rotation <*2.49*>, α_D, of this solution at 20 ± 1°C in a 100-mm cell.

$$\text{Amount (g) of isosorbide } (C_6H_{10}O_4) = \alpha_D \times 2.1978$$

Containers and storage Containers—Tight containers.

Isosorbide Dinitrate

硝酸イソソルビド



$C_6H_8N_2O_8$: 236.14
1,4:3,6-Dianhydro-D-glucitol dinitrate
[*87-33-2*]

Isosorbide Dinitrate contains not less than 95.0% of isosorbide dinitrate ($C_6H_8N_2O_8$), calculated on the anhydrous basis.

Description Isosorbide Dinitrate occurs as white, crystals or crystalline powder. It is odorless or has a faint odor like that of nitric acid.

It is very soluble in *N*,*N*-dimethylformamide and in acetone, freely soluble in chloroform and in toluene, soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

It explodes if heated quickly or subjected to percussion.

Identification **(1)** Dissolve 10 mg of Isosorbide Dinitrate in 1 mL of water, and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS, and allow to stand for 5 to 10 minutes: a brown ring is produced at the zone of contact.

(2) Dissolve 0.1 g of Isosorbide Dinitrate in 6 mL of diluted sulfuric acid (1 in 2) by heating in a water bath. After cooling, add 1 mL of a solution of potassium permanganate (1 in 30), stir well, and heat in a water bath until the color of potassium permanganate disappears. Add 10 mL of 2,4-dinitro-phenylhydrazine TS, and heat in a water bath: an orange precipitate is produced.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +134 – +139° (1 g calculated on the anhydrous basis, ethanol (95), 100 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Isosorbide Dinitrate in 10 mL of acetone: the solution is clear and colorless.

(2) Sulfate <*1.14*>—Dissolve 1.5 g of Isosorbide Dinitrate in 15 mL of *N*,*N*-dimethylformamide, add 60 mL of water, cool, and filter. Wash the filter paper with three 20-mL portions of water, combine the washings with the filtrate, and add water to make 150 mL. To 40 mL of this solution add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Nitrate—Dissolve 50 mg of Isosorbide Dinitrate in 30 mL of toluene, and extract with three 20-mL portions of water. Combine the aqueous layers, and wash with two 20-mL portions of toluene. To the aqueous layer add water to make 100 mL, and use this solution as the sample solution. Pipet 5.0 mL of Standard Nitric Acid Solution and 25 mL of the sample solution in each Nessler tube, and add water to make 50 mL, respectively. To each of them add 60 mg of Griss-Romijin's nitric acid reagent, stir well, allow to stand for 30 minutes, and observe from the side of the Nessler tube: the sample solution has no more color than the standard solution.

(4) Heavy metals <*1.07*>—Dissolve 1.0 g of Isosorbide Dinitrate in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

Water <*2.48*> Not more than 1.5% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Isosorbide Dinitrate in a Kjeldahl flask as described under the Nitrogen Determination <*1.08*>, dissolve in 10 mL of methanol, add 3 g of Devarda's alloy and 50 mL of water, and connect the flask with the distillation apparatus as described under the Nitrogen Determination <*1.08*>. Measure exactly 25 mL of 0.05 mol/L sulfuric acid VS in an absorption flask, add 5 drops of bromocresol green-methyl red TS, and immerse the lower end of the condenser tube in it. Add 15 mL of a solution of sodium hydroxide (1 in 2) through the funnel, cautiously rinse the funnel with 20 mL of water, immediately close the clamp attached to the rubber tubing, then begin the distillation with steam gradually, and continue the distillation until

the distillate measures 100 mL. Remove the absorption flask, rinse the end of the condenser tube with a small quantity of water, and titrate <2.50> the distillate and the rinsings with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red through light red-purple to light blue-green. Perform a blank determination.

Each mL of 0.05 mol/L sulfuric acid VS
= 11.81 mg of $C_6H_8N_2O_8$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Isosorbide Dinitrate Tablets

硝酸イソソルビド錠

Isosorbide Dinitrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$: 236.14).

Method of preparation Prepare as directed under Tablets, with Isosorbide Dinitrate.

Identification Weigh a quantity of powdered Isosorbide Dinitrate Tablets, equivalent to 0.1 g of Isosorbide Dinitrate, add 50 mL of diethyl ether, shake well, and filter. Measure 5 mL of the filtrate, evaporate to dryness cautiously, add 1 mL of water to the residue, and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS, and allow to stand for 5 to 10 minutes: a brown ring is produced at the zone of contact.

Purity Nitrate—Weigh accurately a quantity of powdered Isosorbide Dinitrate Tablets, equivalent to 50 mg of Isosorbide Dinitrate, transfer to a separator, add 30 mL of toluene, shake thoroughly, extract with three 20-mL portions of water, and proceed as directed in Purity (3) under Isosorbide Dinitrate.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Isosorbide Dinitrate Tablets add 1 mL of water, and shake to disintegrate. To this solution add a mixture of water and methanol (1:1) to make exactly V mL so that each mL contains about 0.1 mg of isosorbide dinitrate ($C_6H_8N_2O_8$), and shake for 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of isosorbide dinitrate ($C_6H_8N_2O_8$)
$= M_S \times A_T/A_S \times V \times 1/500$

M_S: Amount (mg) of isosorbide dinitrate for assay taken, calculated on the anhydrous basis

Disintegration <6.09> It meets the requirement.

For Sublingual Tablets, the time limit of the test is 2 minutes, and omit the use of the disk.

Assay Weigh accurately the mass of not less than 20 tablets of Isosorbide Dinitrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of isosorbide dinitrate ($C_6H_8N_2O_8$), add a mixture of water and methanol (1:1) to make exactly 50 mL, and shake for 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of isosorbide dinitrate for assay (separately, determine the water <2.48> in the same manner as Isosorbide Dinitrate), dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of isosorbide dinitrate in each solution.

Amount (mg) of isosorbide dinitrate ($C_6H_8N_2O_8$)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of isosorbide dinitrate for assay taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (11:9).

Flow rate: Adjust so that the retention time of isosorbide dinitrate is about 6 minutes.

System suitability—

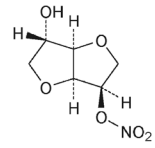
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide dinitrate are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide dinitrate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Isosorbide Mononitrate 70%/Lactose 30%

70%一硝酸イソソルビド乳糖末



$C_6H_9NO_6$: 191.14
1,4:3,6-Dianhydro-D-glucitol 5-nitrate
[*16051-77-7*, Isosorbide mononitrate]

Isosorbide Mononitrate 70%/Lactose 30%, when dried, contains not less than 68.0% and not more than 72.0% of isosorbide mononitrate ($C_6H_9NO_6$).

Description Isosorbide Mononitrate 70%/Lactose 30% occurs as a white, powder, crystalline powder, or masses.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Shake thoroughly 1 g of Isosorbide Mononitrate 70%/Lactose 30% with 30 mL of ethyl acetate, and filter. Wash the residue with a small quantity of ethyl acetate, combine the filtrate and the washings, evaporate to dryness on a water bath, then dry in vacuum at room tem-

perature for 4 hours. Determine the infrared absorption spectrum of the crystals obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum of isosorbide mononitrate: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dry the residue obtained in (1) at 80°C for 2 hours. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum of Lactose Hydrate or the spectrum of Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +116 – +124° (after drying, 1 g, water, 100 mL, 100 mm).

Purity **(1)** Nitrate—Dissolve an exact quantity of Isosorbide Mononitrate 70%/Lactose 30%, equivalent to 50 mg of isosorbide mononitrate ($C_6H_9NO_6$), in water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, to exactly 5 mL of Standard Nitric Acid Solution add water to make exactly 150 mL. Pipet 25 mL of this solution, add water to make exactly 150 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area of nitric acid of each solution by the automatic integration method: the peak area of nitric acid obtained from the sample solution is not larger than the peak area of nitric acid obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with gel type strong basic ion-exchange resin for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 16.0 g of sodium gluconate, 18.0 g of boric acid, 25.0 g of sodium tetraborate decahydrate, and 250 mL of glycerin in water to make 1000 mL. To 20 mL of this solution add 20 mL of 1-butanol, 120 mL of acetonitrile, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of nitric acid is about 5.3 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nitric acid are not less than 800 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitric acid is not more than 2.0%.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Isosorbide Mononitrate 70%/Lactose 30% according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Isosorbide—To an amount of Isosorbide Mononitrate 70%/Lactose 30%, equivalent to 1.0 g of isosorbide mononitrate ($C_6H_9NO_6$), add 10 mL of acetone, shake well, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm. To the residue add 2 mL of acetone and proceed in the same manner, and combine the filtrates. Evaporate the combined filtrate to dryness on a water bath, and further dry the residue in vacuum for 30 minutes. Dissolve the residue in the mobile phase to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas by the automatic integration method: the peak area of isosorbide, having the relative retention time of about 0.2 to isosorbide mononitrate, obtained from the sample solution is not larger than the peak area of isosorbide mononitrate obtained from the standard solution.

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (9:1).

Flow rate: Adjust so that the retention time of isosorbide mononitrate is about 16 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide mononitrate are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide mononitrate is not more than 4.0%.

(4) Related substances—Dissolve an amount of Isosorbide Mononitrate 70%/Lactose 30%, equivalent to 50 mg of isosorbide mononitrate ($C_6H_9NO_6$), in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than isosorbide mononitrate obtained from the sample solution is not larger than 1/2 times the peak area of isosorbide mononitrate obtained from the standard solution, and the total area of the peaks other than isosorbide mononitrate from the sample solution is not larger than the peak area of isosorbide mononitrate from the standard solution. For the area of the peak, having the relative retention time of about 4.5 to isosorbide mononitrate, multiply the relative response factor, 0.62.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of isosorbide mononitrate, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 1 mL of the standard

solution, and add water to make exactly 10 mL. Confirm that the peak area of isosorbide mononitrate obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide mononitrate is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Water ⟨*2.48*⟩ Between 1.0% and 2.0% (0.4 g, direct titration. Use a mixture of methanol for water determination and formamide for water determination (2:1) instead of methanol for water determination).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately an amount of Isosorbide Mononitrate 70%/Lactose 30%, previously dried, equivalent to about 0.2 g of isosorbide mononitrate ($C_6H_9NO_6$), and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution, then add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of isosorbide mononitrate for assay, previously dried, and dissolve in 60 mL of water, add exactly 20 mL of the internal standard solution, then, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of isosorbide mononitrate to that of the internal standard.

Amount (mg) of isosorbide mononitrate ($C_6H_9NO_6$)
$= M_S \times Q_T/Q_S \times 5$

M_S: Amount (mg) of isosorbide mononitrate for assay taken

Internal standard solution—A solution of benzyl alcohol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and methanol (4:1).

Flow rate: Adjust so that the retention time of isosorbide mononitrate is about 4.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isosorbide mononitrate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of isosorbide mononitrate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Isosorbide Mononitrate Tablets

一硝酸イソソルビド錠

Isosorbide Mononitrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$: 191.14).

Method of preparation Prepare as directed under Tablets, with Isosorbide Mononitrate 70%/Lactose 30%.

Identification Shake well a portion of powdered Isosorbide Mononitrate Tablets, equivalent to 50 mg of isosorbide mononitrate ($C_6H_9NO_6$), with 5 mL of acetone, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of isosorbide mononitrate for assay in 1 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (2:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of potassium permanganate in potassium hydroxide TS (1 in 50), and allow to stand for about 50 minutes: the principal spot obtained with the sample solution and the spot obtained with the standard solution are yellow, and their *R*f values are the same.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Isosorbide Mononitrate Tablets add 30 mL of water, allow standing to disintegrate the tablet, and disperse the fine particles with the aid of ultrasonic waves. Add exactly V/10 mL of the internal standard solution, and add water to make V mL so that each mL contains about 0.2 mg of isosorbide mononitrate ($C_6H_9NO_6$). Centrifuge this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of isosorbide mononitrate for assay, previously dried in vacuum (silica gel) for 4 hours, add 30 mL of water and exactly 10 mL of the internal standard solution, then add water to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of isosorbide mononitrate ($C_6H_9NO_6$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of isosorbide mononitrate for assay taken

Internal standard solution—A solution of benzyl alcohol (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Isosorbide Mononitrate Tablets is not less than 85%.

Start the test with 1 tablet of Isosorbide Mononitrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of isosorbide mononitrate

($C_6H_9NO_6$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of isosorbide mononitrate for assay, previously dried in vacuum (silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of isosorbide mononitrate in each solution.

Dissolution rate (%) with respect to the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of isosorbide mononitrate for assay taken
C: Labeled amount (mg) of isosorbide mononitrate ($C_6H_9NO_6$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide mononitrate are not less than 2000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isosorbide mononitrate is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Isosorbide Mononitrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of isosorbide mononitrate ($C_6H_9NO_6$), add 30 mL of water, and disperse the fine particles with the aid of ultrasonic waves. Add exactly 10 mL of the internal standard solution and water to make 50 mL. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of isosorbide mononitrate for assay, previously dried, and dissolve in 30 mL of water, add exactly 10 mL of the internal standard solution, then, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of isosorbide mononitrate to that of the internal standard.

Amount (mg) of isosorbide mononitrate ($C_6H_9NO_6$)
$= M_S \times Q_T/Q_S$

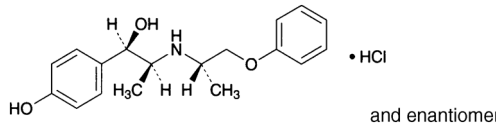
M_S: Amount (mg) of isosorbide mononitrate for assay taken

Internal standard solution—A solution of benzyl alcohol (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 214 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and methanol (4:1).
Flow rate: Adjust so that the retention time of isosorbide mononitrate is about 4.5 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isosorbide mononitrate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of isosorbide mononitrate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Isoxsuprine Hydrochloride

イソクスプリン塩酸塩



$C_{18}H_{23}NO_3.HCl$: 337.84
(1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-{[(2*SR*)-1-phenoxypropan-2-yl]amino}propan-1-ol monohydrochloride
[*579-56-6*]

Isoxsuprine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3.HCl$).

Description Isoxsuprine Hydrochloride occurs as a white, powder or crystalline powder.
It is soluble in formic acid and in methanol, and slightly soluble in water and in ethanol (99.5).
Melting point: about 204°C (with decomposition).
A solution of Isoxsuprine Hydrochloride in methanol (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Isoxsuprine Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Isoxsuprine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(3) Dissolve 0.5 g of Isoxsuprine Hydrochloride in 50 mL of water by warming, and cool: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> Dissolve 0.5 g of Isoxsuprine Hydrochloride in 50 mL of water by warming, and cool: the pH of the solution is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.1 g

of Isoxsuprine Hydrochloride in 10 mL of water, warm if necessary, and cool: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Isoxsuprine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Isoxsuprine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: each peak area other than isoxsuprine obtained from the sample solution is not larger than the peak area of isoxsuprine obtained from the standard solution, and the total area of the peaks other than the peak of isoxsuprine from the sample solution is not larger than 2 times the peak area of isoxsuprine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.3 g of diammonium hydrogen phosphate and 3.2 g of sodium 1-pentane sulfonate in water to make 1000 mL, and adjust to pH 2.5 with phosphoric acid. To 770 mL of this solution add 230 mL of acetonitrile.

Flow rate: Adjust so that the retention time of isoxsuprine is about 18 minutes.

Time span of measurement: About 3 times as long as the retention time of isoxsuprine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of isoxsuprine obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: To 1 mL of the sample solution add 2.5 mL of a solution of methyl parahydroxybenzoate (1 in 25,000) and the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and isoxsuprine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoxsuprine is not more than 2.5%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 1 hour).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Isoxsuprine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.78 mg of $C_{18}H_{23}NO_3.HCl$

Containers and storage Containers—Well-closed containers.

Isoxsuprine Hydrochloride Tablets

イソクスプリン塩酸塩錠

Isoxsuprine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3.HCl$: 337.84).

Method of preparation Prepare as directed under Tablets, with Isoxsuprine Hydrochloride.

Identification To a quantity of powdered Isoxsuprine Hydrochloride Tablets, equivalent to 10 mg of Isoxsuprine Hydrochloride, add 150 mL of water, shake, and then add water to make 200 mL. Centrifuge this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of filtrate, and determine the absorption spectrum of the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 267 nm and 271 nm, and between 272 nm and 276 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add methanol to 1 tablet of Isoxsuprine Hydrochloride Tablets, and shake to disintegrate. Add methanol to make exactly V mL so that each mL contains about 0.4 mg of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3.HCl$). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3.HCl$)
$= M_S \times A_T/A_S \times V \times 1/100$

M_S: Amount (mg) of isoxsuprine hydrochloride for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Isoxsuprine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Isoxsuprine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of isoxsuprine hydrochloride for assay, previously dried at 105°C for 1 hour, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of isoxsuprine in each solution.

Dissolution rate (%) with respect to the labeled amount of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of isoxsuprine hydrochloride for assay taken

C: Labeled amount (mg) of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isoxsuprine are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoxsuprine is not more than 2.0%.

Assay Weigh accurately not less than 20 Isoxsuprine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 40 mg of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl), add 60 mL of methanol, shake for 20 minutes, and then add methanol to make exactly 100 mL. Centrifuge a portion of this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of isoxsuprine hydrochloride for assay, previously dried at 105°C for 1 hour, and dissolve in methanol to make exactly 100 mL. Filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of isoxsuprine in each solution.

Amount (mg) of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of isoxsuprine hydrochloride for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.3 g of diammonium hydrogen phosphate and 3.2 g of sodium 1-pentane sulfonate in water to make 1000 mL, and adjust to pH 2.5 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.

Flow rate: Adjust so that the retention time of isoxsuprine is about 9 minutes.

System suitability—

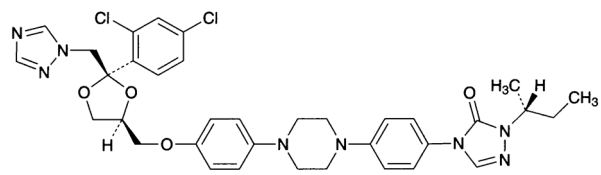
System performance: To exactly 1 mL of the standard solution add the mobile phase to make exactly 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isoxsuprine are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoxsuprine is not more than 1.0%.

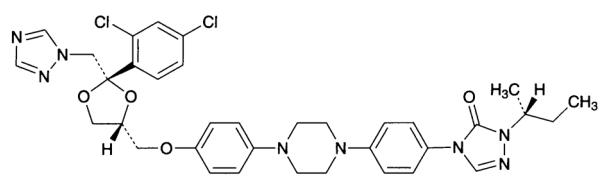
Containers and storage Containers—Well-closed containers.

Itraconazole

イトラコナゾール



and enantiomer



and enantiomer

$C_{35}H_{38}Cl_2N_8O_4$: 705.63

4-(4-{4-[4-({(2*RS*,4*SR*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one
4-(4-{4-[4-({(2*SR*,4*RS*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one
[*84625-61-6*]

Itraconazole contains not less than 98.5% and not more than 101.0% of itraconazole ($C_{35}H_{38}Cl_2N_8O_4$), calculated on the dried basis.

Description Itraconazole occurs as a white powder.

It is soluble in *N*,*N*-dimethylformamide, very slightly soluble in ethanol (99.5), and practically insoluble in water and in 2-propanol.

A solution of Itraconazole in *N*,*N*-dimethylformamide (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Itraconazole in 2-propanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Itraconazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Itraconazole as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 166 – 170°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of

Itraconazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Itraconazole in 10 mL of a mixture of methanol and tetrahydrofuran (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of each peak other than itraconazole obtained from the sample solution is not larger than the peak area of itraconazole obtained from the standard solution. Furthermore, the total area of the peaks other than itraconazole from the sample solution is not larger than 2.5 times the peak area of itraconazole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A solution of tetrabutylammonium hydrogensulfate (17 in 625).

Mobile phase B: Acetonitrile.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	80 → 50	20 → 50
20 – 25	50	50

Flow rate: 1.5 mL per minute.

Time span of measurement: About 2 times as long as the retention time of itraconazole, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 10 mL. Confirm that the peak area of itraconazole obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 1 mg of Itraconazole and 1 mg of miconazole nitrate in 20 mL of the mixture of methanol and tetrahydrofuran (1:1). When the procedure is run with 10 μL of this solution under the above operating conditions, miconazole and itraconazole are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of itraconazole is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Itraconazole, dissolve in 70 mL of a mixture of 2-butanone and acetic acid (100) (7:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.28 mg of $C_{35}H_{38}Cl_2N_8O_4$

Containers and storage Containers—Tight containers.

Japanese Encephalitis Vaccine

日本脳炎ワクチン

Japanese Encephalitis Vaccine is a liquid for injection containing inactivated Japanese encephalitis virus.

It conforms to the requirements of Japanese Encephalitis Vaccine in the Minimum Requirements for Biological Products.

Description Japanese Encephalitis Vaccine is a clear or a slightly whitish turbid and colorless liquid.

Freeze-dried Japanese Encephalitis Vaccine

乾燥日本脳炎ワクチン

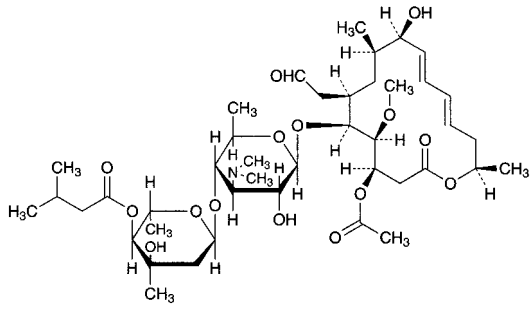
Freeze-dried Japanese Encephalitis Vaccine is a preparation for injection which is dissolved before use. It contains inactivated Japanese encephalitis virus.

It conforms to the requirements of Freeze-dried Japanese Encephalitis Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Japanese Encephalitis Vaccine is a clear or a slightly whitish turbid and colorless liquid on addition of solvent.

Josamycin

ジョサマイシン



$C_{42}H_{69}NO_{15}$: 827.99

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[2,6-dideoxy-4-*O*-(3-methylbutanoyl)-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

[*16846-24-5*]

Josamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces narbonensis* var. *josamyceticus*.

It contains not less than 900 μg (potency) and not more than 1100 μg (potency) per mg, calculated on the dried basis. The potency of Josamycin is expressed as mass (potency) of josamycin ($C_{42}H_{69}NO_{15}$).

Description Josamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in ethanol (99.5), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Josamycin in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin and Josamycin RS in 1 mL of methanol, add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from these solutions: the retention time of the main peak obtained from the sample solution is the same as that of the peak of josamycin obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Josamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 50 mg of Josamycin in 5 mL of methanol, add diluted methanol (1 in 2) to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of josamycin and the related substances by the area percentage method: the amounts of the peaks other than josamycin are not more than 6%, and the total of these peaks is not more than 20%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 119 g of sodium perchlorate monohydrate in water to make 1000 mL, and adjust the pH to 2.5 with 1 mol/L hydrochloric acid TS. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust so that the retention time of josamycin is about 10 minutes.

Time span of measurement: About 4 times as long as the retention time of josamycin, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure 3 mL of the sample solution, add diluted methanol (1 in 2) to make 50 mL, and use this solution as the solution for system suitability test. Measure exactly 2 mL of the solution for system suitability test, and add diluted methanol (1 in 2) to make exactly 20 mL. Confirm that the peak area of josamycin obtained from 10 μL of this solution is equivalent to 8 to 12% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve about 0.05 g of Josamycin in 50 mL of 0.1 mol/L potassium dihydrogen phosphate TS (pH 2.0) and allow to stand at 40°C for 3 hours. Adjust the pH of this solution to 6.8 to 7.2 with 2 mol/L sodium hydroxide TS, and add 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of josamycin S_1, which relative retention time to josamycin is about 0.9, and josamycin is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of josamycin is not more than 1.5%.

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Josamycin RS, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add water to make solutions so that each

mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Josamycin, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of this solution, add water to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Josamycin Tablets

ジョサマイシン錠

Josamycin Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of josamycin ($C_{42}H_{69}NO_{15}$: 827.99).

Method of preparation Prepare as directed under Tablets, with Josamycin.

Identification To a quantity of powdered Josamycin Tablets, equivalent to 10 mg (potency) of Josamycin, add 100 mL of methanol, shake vigorously, and centrifuge. To 5 mL of the supernatant liquid, add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 229 nm and 233 nm.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, in vacuum, 60°C, 3 hours).

Uniformity of dosage units ⟨*6.02*⟩—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Josamycin Tablets, add 5 mL of water, and shake vigorously to disintegrate the tablet. Add methanol and then use ultrasonic waves to disperse the particles, add methanol to make exactly V mL so that each mL contains about 2 mg (potency) of Josamycin, and centrifuge. Pipet 3 mL of the supernatant liquid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, accurately weigh about 50 mg (potency) of Josamycin RS, dissolve in 5 mL of water and methanol to make exactly 25 mL. Pipet 3 mL of this solution, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 231 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩. However, $\overline{X}$ in the formula for calculation of acceptance value is the result of the assay.

$$\text{Amount [mg (potency)] of josamycin } (C_{42}H_{69}NO_{15}) = M_S \times A_T/A_S \times V/25$$

M_S: Amount [mg (potency)] of Josamycin RS taken

Disintegration ⟨*6.09*⟩ Perform the test using the disk: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

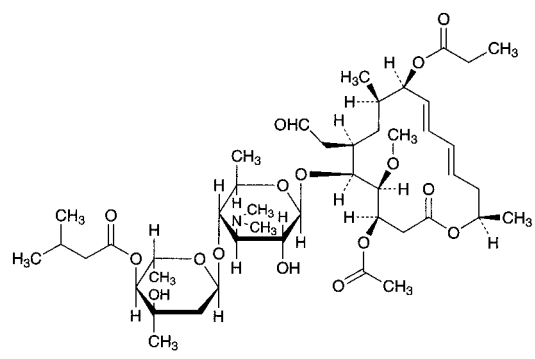
(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Josamycin.

(ii) Sample solutions—Weigh accurately the mass of not less than 20 Josamycin Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 0.3 g (potency) of Josamycin, add 50 mL of methanol, shake vigorously, and add water to make exactly 1000 mL. Take exactly an appropriate amount of this solution, add water to prepare solutions containing 30 μg (potency) and 7.5 μg (potency) per mL, and use these solutions as the high and the low concentration sample solutions, respectively.

Containers and storage Containers—Tight containers.

Josamycin Propionate

ジョサマイシンプロピオン酸エステル



$C_{45}H_{73}NO_{16}$: 884.06
(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[2,6-dideoxy-4-*O*-(3-methylbutanoyl)-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-9-propanoyloxyhexadeca-10,12-dien-15-olide
[*16846-24-5*, Josamycin]

Josamycin Propionate is a derivative of josamycin. It contains not less than 843 μg (potency) and not more than 1000 μg (potency) per mg, calculated on the dried basis. The potency of Josamycin Propionate is expressed as mass (potency) of josamycin ($C_{42}H_{69}NO_{15}$: 827.99).

Description Josamycin Propionate occurs as a white to light yellowish white crystalline powder.

It is very soluble in acetonitrile, freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Josamycin Propionate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin Propionate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin Propionate and Josamycin Propionate RS in 50 mL of diluted acetonitrile (1 in 2), and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and compare the chromatograms obtained from these solutions: the retention time of the peak of

josamycin propionate obtained from the sample solution is the same with that of the peak of josamycin propionate obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Josamycin Propionate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 50 mg of Josamycin Propionate in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of each peak other than josamycin propionate by the area percentage method: the amount of any peak other than josamycin is not more than 6%, and the total of these peaks is not more than 22%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 10 mL of triethylamine add water to make 1000 mL, and adjust the pH to 4.3 with acetic acid (100). To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of josamycin propionate is about 24 minutes.

Time span of measurement: About 3.5 times as long as the retention time of josamycin propionate, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure 3 mL of the sample solution, add the mobile phase to make 50 mL, and use this solution as the solution for system suitability test. Measure exactly 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of josamycin propionate obtained from 10 μL of this solution is equivalent to 8 to 12% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of Josamycin Propionate and 2 mg of josamycin in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, josamycin and josamycin propionate are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of josamycin propionate is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Josamycin Propionate RS, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add 1/15 mol/L phosphate buffer solution (pH 5.6) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 1/15 mol/L phosphate buffer solution (pH 5.6) to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

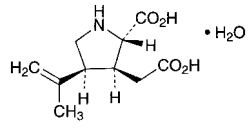
(iv) Sample solutions—Weigh accurately an amount of Josamycin Propionate, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add 1/15 mol/L phosphate buffer solution (pH 5.6) to make exactly 50 mL. Take exactly a suitable amount of this solution, add 1/15 mol/L phosphate buffer solution (pH 5.6) to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Kainic Acid Hydrate

カイニン酸水和物



$C_{10}H_{15}NO_4.H_2O$: 231.25

(2*S*,3*S*,4*S*)-3-(Carboxymethyl)-4-(1-methylethenyl)pyrrolidine-2-carboxylic acid monohydrate

[*487-79-6*, anhydride]

Kainic Acid Hydrate, when dried, contains not less than 99.0% of kainic acid ($C_{10}H_{15}NO_4$: 213.23).

Description Kainic Acid Hydrate occurs as white, crystals or crystalline powder. It is odorless, and has an acid taste.

It is sparingly soluble in water and in warm water, very slightly soluble in acetic acid (100) and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

The pH of a solution of 1.0 g of Kainic Acid Hydrate in 100 mL of water is between 2.8 and 3.5.

Melting point: about 252°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Kainic Acid Hydrate (1 in 5000) add 1 mL of ninhydrin TS, and warm in a water bath at a temperature between 60°C and 70°C for 5 minutes: a yellow color is produced.

(2) Dissolve 50 mg of Kainic Acid Hydrate in 5 mL of acetic acid (100), and add 0.5 mL of bromine TS: the color of bromine disappears immediately.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −13 – −17° (0.5 g, water, 50 mL, 200 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g

of Kainic Acid Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Take 0.5 g of Kainic Acid Hydrate in a platinum crucible, dissolve in 5 mL of sodium carbonate TS, and evaporate on a water bath to dryness. Heat the crucible slowly at first, and then ignite until the sample is almost incinerated. After cooling, add 12 mL of dilute nitric acid to the residue, dissolve by warming, and filter. Wash the residue with 15 mL of water, combine the washings and the filtrate, and add water to make 50 mL. Perform the test using this solution as the test solution.

Control solution: Add 5 mL of sodium carbonate TS to 0.30 mL of 0.01 mol/L hydrochloric acid VS, and proceed as directed above (not more than 0.021%).

(3) Sulfate <*1.14*>—Dissolve 0.5 g of Kainic Acid Hydrate in 40 mL of water by warming. Cool, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Take 0.25 g of Kainic Acid Hydrate, and perform the test. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of Kainic Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <*1.11*>—Dissolve 1.0 g of Kainic Acid Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Amino acid and other imino acid—Dissolve 0.10 g of Kainic Acid Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <*2.03*> with these solutions. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant liquid of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry the plate at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> 6.5 – 8.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Kainic Acid Hydrate, previously dried, and dissolve in 50 mL of warm water, cool and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 21.32 mg of $C_{10}H_{15}NO_4$

Containers and storage Containers—Tight containers.

Kainic Acid and Santonin Powder

カイニン酸·サントニン散

Kainic Acid and Santonin Powder contains not less than 9.0% and not more than 11.0% of santonin ($C_{15}H_{18}O_3$: 246.30), and not less than 1.80% and not more than 2.20% of kainic acid hydrate ($C_{10}H_{15}NO_4.H_2O$: 231.25).

Method of preparation

Santonin	100 g
Kainic Acid Hydrate	20 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Kainic Acid and Santonin Powder occurs as a white powder.

Identification **(1)** Shake 1 g of Kainic Acid and Santonin Powder with 10 mL of chloroform, and filter [use the residue for the test (2)]. Distil off the chloroform of the filtrate, and dissolve the residue in 2 mL of potassium hydroxide-ethanol TS: a red color is produced (santonin).

(2) Shake the residue obtained in (1) with 20 mL of warm water, filter, and to 1 mL of the filtrate add 10 mL of water and 1 mL of ninhydrin-L-ascorbic acid TS. Warm in a water bath between 60°C and 70°C for 5 minutes: a yellow color is produced (kainic acid).

Assay **(1)** Santonin—Weigh accurately about 0.25 g of Kainic Acid and Santonin Powder and about 25 mg of santonin for assay, add 20 mL each of ethanol (95), shake thoroughly for 5 minutes, and filter. Wash the residue with three 10-mL portions of ethanol (95), and filter. Combine the filtrate and the washings, and add ethanol (95) to make exactly 50 mL. Pipet 2 mL each of these solutions, add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of santonin ($C_{15}H_{18}O_3$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of santonin for assay taken

(2) Kainic acid—Weigh accurately about 1.25 g of Kainic Acid and Santonin Powder, add 20 mL of diluted pyridine (1 in 10), shake thoroughly for 5 minutes, and filter. Wash the residue with three 10-mL portions of diluted pyridine (1 in 10), and filter. Combine the filtrate and the washings, and add diluted pyridine (1 in 10) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted pyridine (1 in 10) to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve about 25 mg of kainic acid hydrate for assay, previously dried at 105°C for 4 hours and accurately weighed, in diluted pyridine (1 in 10) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted pyridine (1 in 10) to make exactly 25 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution, add 2 mL of ninhydrin-L-ascorbic acid TS, and heat on a water bath for 30 minutes. After

cooling immediately, shake vigorously for 2 minutes, add water to make exactly 20 mL, and allow to stand for 15 minutes. Determine the absorbances, A_T and A_S, of these solutions at 425 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the solution prepared in the same manner with 2 mL of diluted pyridine (1 in 10) instead of the sample solution as the blank.

Amount (mg) of kainic acid hydrate ($C_{10}H_{15}NO_4.H_2O$)
$= M_S \times A_T/A_S \times 1.085$

M_S: Amount (mg) of kainic acid hydrate for assay taken

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Kallidinogenase

カリジノゲナーゼ

[*9001-01-8*]

Kallidinogenase is an enzyme obtained from healthy porcine pancreas, and has kinin-releasing activity based on cleavage of kininogen.

It contains not less than 25 Kallidinogenase Units per mg. Usually, it is diluted with Lactose Hydrate or the like.

Kallidinogenase contains not less than 90% and not more than 110% of the labeled Units.

Description Kallidinogenase occurs as a white to light brown powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Kallidinogenase (1 in 300) is between 5.5 and 7.5.

Identification (1) Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, and dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to prepare a solution containing 10 Kallidinogenase Units per mL. Pipet 5 mL of this solution, and add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution (pH 7.0) separately to each test tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the sample solutions 1 and 2. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution (pH 7.0) separately to each tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the sample solutions 3 and 4. Then, pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at 30.0 ± 0.5°C for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the sample solution 1 warmed at 30.0 ± 0.5°C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30.0 ± 0.5°C as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank, and determine the absorbances at 405 nm, $A_{1\text{-}2}$ and $A_{1\text{-}6}$, of this solution, after having allowed it to stand for exactly 2 and 6 minutes. Perform the same test with the sample solutions 2, 3 and 4, and determine the absorbances, $A_{2\text{-}2}$, $A_{2\text{-}6}$, $A_{3\text{-}2}$, $A_{3\text{-}6}$, $A_{4\text{-}2}$ and $A_{4\text{-}6}$, of these solutions. Calculate I by using the following equation: the value of I does not exceed 0.2.

$$I = \frac{(A_{1\text{-}6} - A_{1\text{-}2}) - (A_{3\text{-}6} - A_{3\text{-}2})}{(A_{2\text{-}6} - A_{2\text{-}2}) - (A_{4\text{-}6} - A_{4\text{-}2})}$$

(2) Pipet 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at 30.0 ± 0.5°C for 5 minutes, place in a 1-cm cell, add exactly 0.1 mL of the sample solution obtained in the Assay, and start simultaneously a chronograph. Perform the test at 30.0 ± 0.5°C as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the change of the absorbance at 253 nm for 4 to 6 minutes. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Add exactly 0.1 mL of this solution to exactly 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at 30.0 ± 0.5°C for 5 minutes, and use this solution as the blank. If the rate of change in the absorbance remains constant, determine the change of absorbance per 1 minute, A, and calculate R by using the following equation: the value of R is between 0.12 and 0.16.

$$R = A/0.0383 \times 1/(a \times b)$$

a: Amount (mg) of Kallidinogenase in 1 mL of the sample solution

b: Amount (Unit) of kallidinogenase in 1 mg of Kallidinogenase obtained in the Assay

Specific activity Perform the test with Kallidinogenase as directed under Nitrogen Determination <*1.08*> to determine the nitrogen content, convert 1mg of nitrogen (N:14.01) into 6.25 mg of protein, and calculate the specific activity using the amount (Units) of Kallidinogenase obtained in the Assay: it is not less than 100 Kallidinogenase Units per 1mg of protein.

Purity (1) Fat—To 1.0 g of Kallidinogenase add 20 mL of diethyl ether, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue is not more than 1 mg.

(2) Kininase—

(i) Bradykinin solution: Weigh an appropriate amount of bradykinin, and dissolve in gelatin-phosphate buffer solution (pH 7.4) to prepare a solution containing 0.200 μg of bradykinin per mL.

(ii) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase according to the labeled unit, dissolve in gelatin-phosphate buffer solution (pH 7.4) to make a solution containing 1 unit of kallidinogenase per mL.

(iii) Sample solution: Pipet 0.5 mL of bradykinin solution, warm at 30 ± 0.5°C for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at 30 ± 0.5°C for 5 minutes, and mix immediately. After allow this solution to stand at 30 ± 0.5°C for exactly 150 seconds, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution (pH 8.0), and mix. Pipet 0.1 mL of this solution, add exactly 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution, and mix. Pipet 0.2 mL of this solution, add exactly 0.6 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iv) Control solution: Proceed with 0.5 mL of gelatin-phosphate buffer solution (pH 7.4) as described in (iii), and use the solution so obtained as the control solution.

(v) Procedure: Add 0.1 mL of anti-bradykinin antibody TS to anti-rabbit antibody-coated wells of a 96-well microplate, shake, and allow to stand at a constant temperature of about 25°C for 1 hour. Remove the anti-bradykinin antibody TS, add 0.3 mL of phosphate buffer solution for microplate washing to the wells, then remove. Repeat this procedure 3 times, take off the washings thoroughly, then add 100 μL each of the sample solution and control solution, and 50 μL of gelatin-phosphate buffer solution (pH 7.0), shake, and allow to stand at a constant temperature of about 25°C for 1 hour. Then add 50 μL of peroxidase-labeled bradykinin TS, shake, and allow to stand in a cold place for a night. Take off the solution, add 0.3 mL of phosphate buffer solution for microplate washing, and remove. Repeat this procedure more 4 times, take off the washings thoroughly, add 100 μL of substrate solution for peroxidase determination, and allow to stand at a constant temperature of about 25°C for exactly 30 minutes while protecting from light. Then add 100 μL of diluted sulfuric acid (23 in 500), shake, and determine the absorbance at 490 – 492 nm. Separately, dissolve a suitable amount of bradykinin in gelatin-phosphate buffer solution (pH 7.0) to make solutions containing exactly 100 ng, 25 ng, 6.25 ng, 1.56 ng, 0.39 ng and 0.098 ng of bradykinin per mL, and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3), the standard solution (4), the standard solution (5) and the standard solution (6), respectively. Use 1 mL of gelatin-phosphate buffer solution (pH 7.0) as the standard solution (7). To each of the well add 50 μL each of the standard solutions and 100 μL of trichloroacetic acid-gelatin-tris buffer solution, and proceed in the same manner as for the sample solution and for the control solution.

Prepare the standard curve from the amounts of bradykinin in the standard solutions and their absorbances, and calculate the amount of bradykinin, B_T (pg) and B_S (pg), of the sample solution and the control solution.

The absorbance is usually determined by using a spectrophotometer for microplate. Since the wells are used as the cell for absorbance determination, take care for dirt and scratch of the well. Light pass length of the well is changeable by the amount of the liquid, exact addition of the liquid is necessary.

(vi) Judgment: The value R calculated by the following equation is not less than 0.8.

$$R = B_T/B_S$$

(3) Trypsin-like substances—Pipet 4 mL of the sample stock solution prepared for the Assay, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at 30 ± 0.5°C for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the sample solution, warmed at 30 ± 0.5°C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30 ± 0.5°C as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank, and determine the absorbances at 405 nm, A_2 and A_6, of this solution after having allowed it to stand for exactly 2 and 6 minutes. Separately, pipet 4 mL of the sample stock solution prepared for the Assay, add 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL, and use this solution as the control solution. Perform the same test with the control solution, and determine the absorbances, A'_2 and A'_6. Calculate T by using the following equation: the value of T does not exceed 0.05.

$$T = \{(A'_6 - A'_2) - (A_6 - A_2)\}/(A'_6 - A'_2)$$

(4) Protease—Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to prepare a solution containing 1 Kallidinogenase Unit per mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, place in a test tube, and allow to stand at 35 ± 0.5°C for 5 minutes. Then, pipet 5 mL of substrate TS for kallidinogenase assay (3), previously warmed to 35 ± 0.5°C, add quickly to the sample solution in the test tube, and allow to stand at 35 ± 0.5°C for exactly 20 minutes. Then add exactly 5 mL of trichloroacetic acid TS, shake well, allow to stand at room temperature for 1 hour, and filter through a membrane filter (5 μm in pore size). Discard the first 3 mL of the filtrate, and determine the absorbance, A, of the subsequent filtrate at 280 nm within 2 hours as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS, shake well, and add exactly 5 mL of the substrate TS for kallidinogenase assay (3). Proceed in the same manner as described for the sample solution, and determine the absorbance, A_0, of this solution. Calculate the value of (A–A_0): it is not more than 0.2.

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 3% (0.5 g, 650 – 750°C).

Kinin-releasing activity

(i) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase, according to the labeled unit, dissolve in 0.02 mol/L phosphate buffer solution (pH 8.0) to make a solution containing 0.1 unit of kallidinogenase per mL. Perform this procedure by using glassware.

(ii) Sample solution: Pipet 0.5 mL of kininogen TS, warm at 30 ± 0.5°C for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at 30 ± 0.5°C for 5 minutes, and mix immediately. After allow this solution to stand at 30 ± 0.5°C for exactly 2 minutes, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution (pH 8.0), and shake. Pipet 0.1 mL of this solution, add exactly 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iii) Procedure: Perform the test with the sample solution as directed in the Purity (2), and determine the amount, B (pg), of kinin per well. The kinin-releasing activity per 1 unit of Kallidinogenase calculated by the following equation is not less than 500 ng bradykinin equivalent/minute/unit.

Kinin-releasing activity (ng bradykinin equivalent/minute/unit) per 1 unit of Kallidinogenase = $B \times 4.8$

Assay Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to prepare a solution containing about 10 Kallidinogenase Units per mL, and use this solution as the sample stock solution. Pipet 4 mL of the sample stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution (pH 7.0)

to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at 30 ± 0.5°C for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the sample solution, warmed at 30 ± 0.5°C for 5 minutes, and start simultaneously a chronograph. Perform the test at 30 ± 0.5°C as directed under the Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank, and determine the absorbances at 405 nm, A_{T2} and A_{T6}, of this solution after allowing to stand for exactly 2 and 6 minutes. Separately, dissolve Kallidinogenase RS in 0.05 mol/L phosphate buffer solution (pH 7.0) to make a solutin so that each mL contains exactly 10 Units, and use this solution as the standard stock solution. Pipet 4 mL of the stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL, and use this solution as the standard solution. Take exactly 0.5 mL of the standard solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, A_{S2} and A_{S6}, of the solution after allowing to stand for exactly 2 and 6 minutes. Separately, take exactly 1 mL of the trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Pipet 0.5 mL of this solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, A_{O2} and A_{O6}, of the solution after allowing to stand for exactly 2 and 6 minutes.

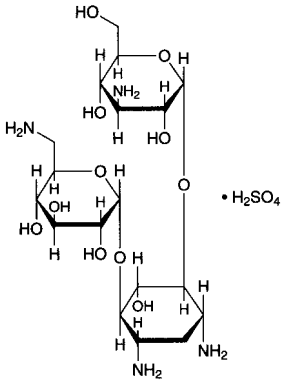
$$\text{Units per 1 mg of Kallidinogenase} = \frac{(A_{T6} - A_{T2}) - (A_{O6} - A_{O2})}{(A_{S6} - A_{S2}) - (A_{O6} - A_{O2})} \times \frac{M_S}{a} \times \frac{1}{b}$$

M_S: Amount (Units) of Kallidinogenase RS taken
a: Volume (mL) of the standard stock solution
b: Amount (mg) of Kallidinogenase in 1 mL of the sample stock solution

Cantainers and storage Containers—Tight containers.

Kanamycin Monosulfate

カナマイシン一硫酸塩



$C_{18}H_{36}N_4O_{11}.H_2SO_4$: 582.58
3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine monosulfate
[*25389-94-0*]

Kanamycin Monosulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces kanamyceticus*.

It contains not less than 750 μg (potency) and not more than 832 μg (potency) per mg, calculated on the dried basis. The potency of Kanamycin Monosulfate is expressed as mass (potency) of kanamycin ($C_{18}H_{36}N_4O_{11}$: 484.50).

Description Kanamycin Monosulfate occurs as a white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 50 mg of Kanamycin Monosulfate in 3 mL of water, and add 6 mL of anthrone TS: a blue-purple color develops.

(2) Dissolve 20 mg each of Kanamycin Monosulfate and Kanamycin Monosulfate RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant layer of a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a purple-brown color and the same *R*f value.

(3) To a solution of Kanamycin Monosulfate (1 in 5) add 1 drop of barium chloride TS: a white precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +112 – +123° (0.2 g calculated on the dried basis, water, 20 mL, 100 mm).

Sulfuric acid Weigh accurately about 0.25 g of Kanamycin Monosulfate, dissolve in 100 mL of water, adjust the pH to 11.0 with ammonia solution (28), add exactly 10 mL of 0.1 mol/L barium chloride VS, and titrate <*2.50*> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution, blue-purple, disappears (indicator: 0.5 mg of phthalein purple). At a near of the end-point add 50 mL of ethanol (99.5). Perform a blank determination in the same manner. The amount of sulfuric acid (SO_4) is not less than 15.0% and not more than 17.0%, calculated on the dried basis.

$$\text{Each mL of 0.1 mol/L barium chloride VS} = 9.606 \text{ mg of } SO_4$$

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.30 g of Kanamycin Monosulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 45 mg of Kanamycin Monosulfate RS in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

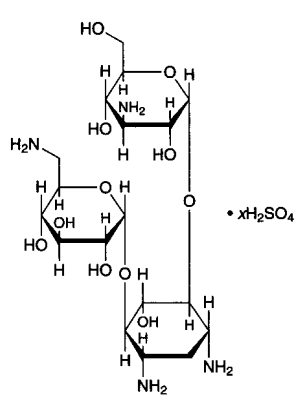
(iii) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution (pH 6.0) (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 and 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kanamycin Monosulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Kanamycin Sulfate

カナマイシン硫酸塩



$C_{18}H_{36}N_4O_{11}.xH_2SO_4$

3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine sulfate

[*133-92-6*]

Kanamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces kanamyceticus*.

It contains not less than 690 μg (potency) and not more than 740 μg (potency) per mg, calculated on the dried basis. The potency of Kanamycin Sulfate is expressed as mass (potency) of kanamycin ($C_{18}H_{36}N_4O_{11}$: 484.50).

Description Kanamycin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 20 mg each of Kanamycin Sulfate and Kanamycin Monosulfate RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a purple-brown color and the same *R*f value.

(2) A solution of Kanamycin Sulfate (1 in 10) responds to the Qualitative Test ⟨*1.09*⟩ (1) for sulfate.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +103 – +115° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Kanamycin Sulfate in 20 mL of water is between 6.0 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.5 g of Kanamycin Sulfate in 5 mL of water: the solution is clear. Determine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: not more than 0.15.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Kanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Kanamycin Sulfate according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.30 g of Kanamycin Sulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 9.0 mg of Kanamycin Monosulfate RS in water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer having pH 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer

solution (pH 6.0) (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kanamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Kaolin

カオリン

Kaolin is a native, hydrous aluminum silicate.

Description Kaolin occurs as white or nearly white, fragmentary masses or powder. It has a slightly clay-like odor.

It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It is insoluble in dilute hydrochloric acid and in sodium hydroxide TS.

When moistened with water, it darkens and becomes plastic.

Identification **(1)** Heat 1 g of Kaolin with 10 mL of water and 5 mL of sulfuric acid in a porcelain dish, and evaporate the mixture nearly to dryness. Cool, add 20 mL of water, boil for 2 to 3 minutes, and filter: the color of the residue is gray.

(2) The filtrate obtained in (1) responds to the Qualitative Tests <*1.09*> (1), (2) and (4) for aluminum salt.

Purity **(1)** Acid or alkali—Add 25 mL of water to 1.0 g of Kaolin, agitate thoroughly, and filter: the pH <*2.54*> of the filtrate is between 4.0 and 7.5.

(2) Acid-soluble substances—Add 20 mL of dilute hydrochloric acid to 1.0 g of Kaolin, agitate for 15 minutes, and filter. Evaporate 10 mL of the filtrate to dryness, and heat strongly between 450°C and 550°C to constant mass: the mass of the ignited residue is not more than 10 mg.

(3) Carbonate—Stir 1.0 g of Kaolin with 5 mL of water, then add 10 mL of diluted sulfuric acid (1 in 2): no effervescence occurs.

(4) Heavy metals <*1.07*>—Boil 1.5 g of Kaolin gently with 50 mL of water and 5 mL of hydrochloric acid for 20 minutes with frequent agitation, cool, centrifuge, and separate the supernatant liquid. Wash the precipitate twice with 10 mL of water, centrifuge each time, and combine the supernatant liquid and the washings. Add dropwise ammonia solution (28) to this solution until a slight precipitate occurs, then add dilute hydrochloric acid dropwise while agitating strongly to complete solution. Add 0.45 g of hydroxylammonium chloride, and heat. Cool, add 0.45 g of sodium acetate trihydrate and 6 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Combine the filtrate and the washings, and add water to make 150 mL. Perform the test using 50 mL of this solution as the test solution. To 2.5 mL of Standard Lead Solution add 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than 50 ppm).

(5) Iron <*1.10*>—Add 10 mL of dilute hydrochloric acid to 40 mg of Kaolin, and heat for 10 minutes with shaking in a water bath. After cooling, add 0.5 g of L-tartaric acid, dissolve with shaking, prepare the test solution with this solution according to Method 2, and perform the test according to Method B. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(6) Arsenic <*1.11*>—Add 5 mL of water and 1 mL of sulfuric acid to 1.0 g of Kaolin, and heat on a sand bath until white fumes begin to evolve. Cool, and add water to make 5 mL. Perform the test with this solution as the test solution (not more than 2 ppm).

(7) Foreign matter—Place 5 g of Kaolin in a beaker, add 100 mL of water, stir, and decant to leave sand. Repeat this procedure several times with 100-mL portions of water: no sandy residue remains.

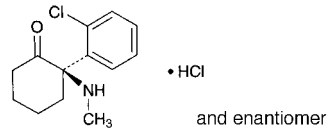
Loss on ignition <*2.43*> Not more than 15.0% (1 g, 600°C, 5 hours).

Plasticity Add 7.5 mL of water to 5.0 g of Kaolin, and agitate thoroughly: the resultant mass has no remarkable fluidity.

Containers and storage Containers—Well-closed containers.

Ketamine Hydrochloride

ケタミン塩酸塩



and enantiomer

$C_{13}H_{16}ClNO.HCl$: 274.19

(2*RS*)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone monohydrochloride

[*1867-66-9*]

Ketamine Hydrochloride, when dried, contains not less than 99.0% of ketamine hydrochloride ($C_{13}H_{16}ClNO.HCl$).

Description Ketamine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in water and in methanol, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

A solution of Ketamine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 258°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ketamine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 3000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketamine Hydrochloride, previously dried, as directed in the

potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ketamine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> (2) for chloride.

Absorbance <2.24> $E^{1\%}_{1\,cm}$ (269 nm): 22.0 – 24.5 (after drying, 30 mg, 0.1 mol/L hydrochloric acid TS, 100 mL).

pH <2.54> Dissolve 1.0 g of Ketamine Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ketamine Hydrochloride in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ketamine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ketamine Hydrochloride, according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.5 g of Ketamine Hydrochloride in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and isopropylamine (49:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, dry the plate, and then spray evenly hydrogen peroxide TS: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more then 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

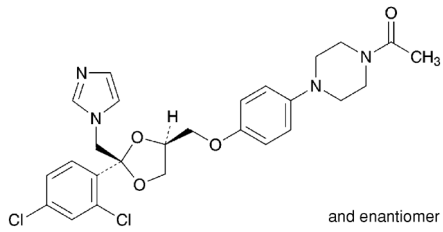
Assay Weigh accurately about 0.5 g of Ketamine Hydrochloride, previously dried, dissolve in 1 mL of formic acid, add 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.42 mg of $C_{13}H_{16}ClNO.HCl$

Containers and storage Containers—Tight containers.

Ketoconazole

ケトコナゾール



$C_{26}H_{28}Cl_2N_4O_4$: 531.43
1-Acetyl-4-(4-{[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazine
[*65277-42-1*]

Ketoconazole, when dried, contains not less than 99.0% and not more than 101.0% of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$).

Description Ketoconazole occurs as a white to light yellowish white powder.

It is soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Ketoconazole in methanol (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Ketoconazole in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketoconazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Ketoconazole as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 148 – 152°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Ketoconazole according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related Substances—Dissolve 0.10 g of Ketoconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than ketoconazole obtained from the sample solution is not larger than 2/5 times the peak area of ketoconazole obtained from the standard solution, and the total area of the peaks other than ketoconazole from the sample solution is not larger than the peak area of ketoconazole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Acetonitrile for liquid chromatography.

Mobile phase B: A solution of tetrabutylammonium hydrogensulfate (17 in 5000).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	5 → 50	95 → 50
10 – 15	50	50

Flow rate: 2.0 mL per minute.

Time span of measurement: For 15 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of ketoconazole obtained from 10 μL of this solution is equivalent to 7 to 13% of that of ketoconazole obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ketoconazole are not less than 40,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ketoconazole is not more than 2.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ketoconazole, previously dried, dissolve in 70 mL of a mixture of 2-butanone and acetic acid (100) (7:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.57 mg of $C_{26}H_{28}Cl_2N_4O_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ketoconazole Cream

ケトコナゾールクリーム

Ketoconazole Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$: 531.43).

Method of preparation Prepare as directed under Creams, with Ketoconazole.

Identification To a quantity of Ketoconazole Cream, equivalent to 0.1 g of Ketoconazole, add 20 mL of 2-propanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of ketoconazole in 5 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28) (40:40:25:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same *R*f value as the spot obtained from the standard solution.

Assay Weigh accurately an amount of Ketoconazole Cream, equivalent to about 25 mg of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of ketoconazole for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard.

Amount (mg) of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of ketoconazole for assay taken

Internal standard solution—A solution of xanthone in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To ammonium acetate solution (1 in 200) add acetic acid (100) to adjust the pH to 5.0. To 250 mL of this solution add 750 mL of methanol.

Flow rate: Adjust so that the retention time of ketoconazole is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ketoconazole are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ketoconazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ketoconazole Lotion

ケトコナゾールローション

Ketoconazole Lotion is an emulsion lotion.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$: 531.43).

Method of preparation Prepare as directed under Lotions, with Ketoconazole.

Description Ketoconazole Lotion occurs as a white emulsion.

Identification Shake well and take an amount of Ketoconazole Lotion, equivalent to 0.1 g of Ketoconazole, add 20 mL of 2-propanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of ketoconazole in 5 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28) (40:40:25:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same *R*f value as the spot obtained from the standard solution.

Assay Shake well and weigh accurately an amount of Ketoconazole Lotion, equivalent to about 25 mg of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of ketoconazole for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard.

$$\text{Amount (mg) of ketoconazole } (C_{26}H_{28}Cl_2N_4O_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of ketoconazole for assay taken

Internal standard solution—A solution of xanthone in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To ammonium acetate solution (1 in 200) add acetic acid (100) to adjust the pH to 5.0. To 250 mL of this solution add 750 mL of methanol.

Flow rate: Adjust so that the retention time of ketoconazole is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ketoconazole are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ketoconazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ketoconazole Solution

ケトコナゾール液

Ketoconazole Solution is a liquid for external use.

Ketoconazole Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$: 531.43).

Method of preparation Prepare as directed under Liquids and Solutions for Cutaneous Application, with Ketoconazole.

Description Ketoconazole Solution is a clear liquid.

Identification To a volume of Ketoconazole Solution, equivalent to 10 mg of Ketoconazole, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of ketoconazole in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28) (40:40:30:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same *R*f value as the spot obtained from the standard solution.

pH Being specified separately when the drug is granted approval based on the Law.

Assay To an exact amount of Ketoconazole Solution, equivalent to about 10 mg of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), add exactly 5 mL of the internal standard solution, and add 15 mL of methanol. To 1 mL of this solution add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ketoconazole for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 20 mL. Take 1 mL of this solution, add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard.

$$\text{Amount (mg) of ketoconazole } (C_{26}H_{28}Cl_2N_4O_4) = M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of ketoconazole for assay taken

Internal standard solution—A solution of bifonazole in methanol (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of diisopropylamine in methanol (1 in 500), ammonium acetate solution (1 in 200) and acetic acid (100) (1800:600:1).

Flow rate: Adjust so that the retention time of ketoconazole is about 11 minutes.

System suitability—

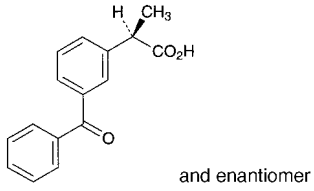
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ketoconazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ketoconazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ketoprofen

ケトプロフェン



and enantiomer

$C_{16}H_{14}O_3$: 254.28

(2*RS*)-2-(3-Benzoylphenyl)propanoic acid

[*22071-15-4*]

Ketoprofen, when dried, contains not less than 99.0% and not more than 100.5% of ketoprofen ($C_{16}H_{14}O_3$).

Description Ketoprofen occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95) and in acetone, and practically insoluble in water.

A solution of Ketoprofen in ethanol (99.5) (1 in 100) shows no optical rotation.

It is colored to pale yellow by light.

Identification **(1)** Determine the absorption spectrum of a solution of Ketoprofen in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketoprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 94 – 97°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ketoprofen in 10 mL of aceton: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixure of 0.6 mL of Cobalt (II) Chloride CS and 2.4 mL of Iron (III) Chloride CS add diluted hydrochloric acid (1 in 10) to make 10 mL. To 5.0 mL of this solution add diluted hydrochloric acid (1 in 10) to make 100 mL.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Ketoprofen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure with a minimum of exposure to light, using light-resistant vessels. Dissolve 20 mg of Ketoprofen in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention time of about 1.5 and about 0.3 to ketoprofen from the sample solution, are not larger than 4.5 times and not larger than 2 times the peak area of ketoprofen from the standard solution, respectively, the area of the peak other than ketoprofen and the peaks mentioned above from the sample solution is not larger than the peak area of ketoprofen from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of ketoprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 233 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 68.0 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with phosphoric acid. To 20 mL of this solution add 430 mL of acetonitrile and 550 mL of water.

Flow rate: Adjust so that the retention time of ketoprofen is about 7 minutes.

Time span of measurement: About 7 times as long as the retention time of ketoprofen.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ketoprofen obtained with 20 μL of this solution is equivalent to 9 to 11% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ketoprofen are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ketoprofen is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, 60°C, 24 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

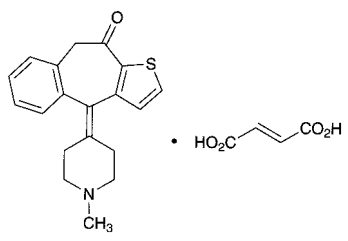
Assay Weigh accurately about 0.3 g of Ketoprofen, previously dried, dissolve in 25 mL of ethanol (95), add 25 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.43 mg of $C_{16}H_{14}O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ketotifen Fumarate

ケトチフェンフマル酸塩



$C_{19}H_{19}NOS.C_4H_4O_4$: 425.50
4-(1-Methylpiperidin-4-ylidene)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10(9*H*)-one monofumarate
[*34580-14-8*]

Ketotifen Fumarate, when dried, contains not less than 99.0% and not more than 101.0% of ketotifen fumarate ($C_{19}H_{19}NOS.C_4H_4O_4$).

Description Ketotifen Fumarate occurs as a white to light yellowish white crystalline powder.

It is sparingly soluble in methanol and in acetic acid (100), and slightly soluble in water, in ethanol (99.5) and in acetic anhydride.

Melting point: about 190°C (with decomposition).

Identification **(1)** Prepare the test solution with 30 mg of Ketotifen Fumarate as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩ using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ for sulfate.

(2) Determine the absorption spectrum of a solution of Ketotifen Fumarate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ketotifen Fumarate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 0.6 g of Ketotifen Fumarate in 2.5 mL of sodium carbonate TS in a crucible, heat on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of sodium carbonate TS, the used amount of diluted nitric acid (3 in 10) for the neutralization, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ketotifen Fumarate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Ketotifen Fumarate in 10 mL of a mixture of methanol and ammonia TS (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of methanol and ammonia TS (99:1) to make exactly 25 mL. Pipet 1 mL of this solution, add a mixture of methanol and ammonia TS (99:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (90:10:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying and then hydrogen peroxide TS on the plate: the number of the spot other than the principal spot obtained from the sample solution is not more than four, and they are not more intense than the spot obtained from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Ketotifen Fumarate, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

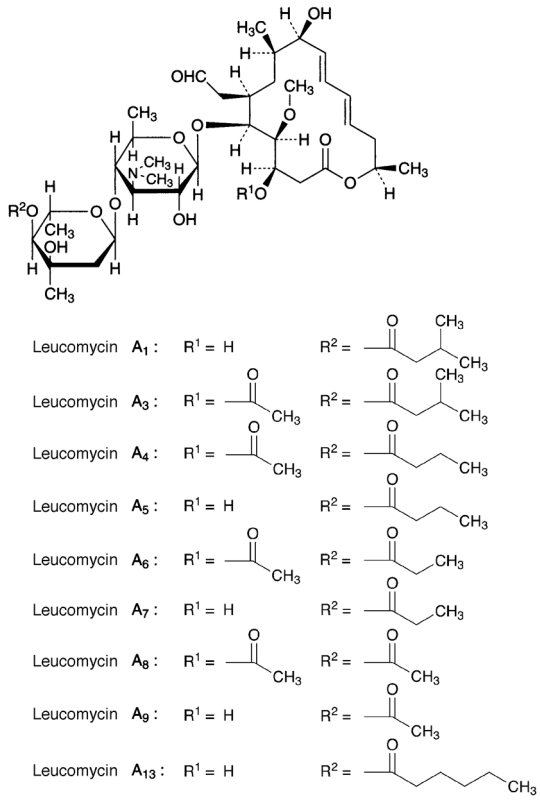
Each mL of 0.1 mol/L perchloric acid VS
= 42.55 mg of $C_{19}H_{19}NOS.C_4H_4O_4$

Containers and storage Containers—Tight containers.

Kitasamycin

Leucomycin

キタサマイシン



(Leucomycins A_1, A_5, A_7, A_9 and A_{13})
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Leucomycin A_1 : acyl = 3-methylbutanoyl
Leucomycin A_5 : acyl = butanoyl
Leucomycin A_7 : acyl = propanoyl
Leucomycin A_9 : acyl = acetyl
Leucomycin A_{13}: acyl = hexanoyl

(Leucomycins A_3, A_4, A_6 and A_8)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[4-*O*-acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Leucomycin A_3: acyl = 3-methylbutanoyl
Leucomycin A_4: acyl = butanoyl
Leucomycin A_6: acyl = propanoyl
Leucomycin A_8: acyl = acetyl

[*1392-21-8*, Kitasamycin]

Kitasamycin is a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces kitasatoensis*.

It contains not less than 1450 μg (potency) and not more than 1700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.93). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin occurs as a white to light yellow-white powder.

It is very soluble in acetonitrile, in methanol and in ethanol (95), and practically insoluble in water.

Identification Determine the absorption spectrum of a solution of Kitasamycin in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Content ratio of the active principle Dissolve 20 mg of Kitasamycin in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 by the area percentage method: the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 are 40 to 70%, 5 to 25% and 3 to 12%, respectively. Relative retention times of leucomycin A_4 and leucomycin A_1 to leucomycin A_5 are about 1.2 and about 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To a volume of a solution of ammonium acetate (77 in 5000) add diluted phosphoric acid (1 in 150) to adjust to pH 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust so that the retention time of leucomycin A_5 is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin A_5.

System suitability—

System performance: Dissolve about 20 mg each of Leucomycin A_5 RS and Josamycin RS in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A_5 and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A_5 is not more than 1.0%.

Water <*2.48*> Not more than 3.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Leucomycin A_5 RS equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100

mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

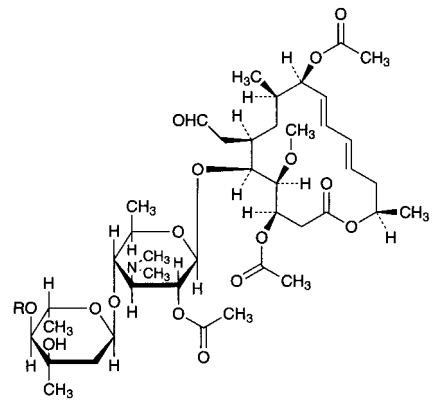
(iv) Sample solutions—Weigh accurately an amount of Kitasamycin equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of the solution, add phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

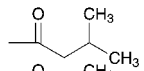
Containers and storage Containers—Tight containers.

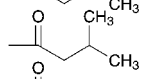
Kitasamycin Acetate

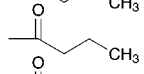
Leucomycin Acetate

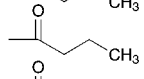
キタサマイシン酢酸エステル

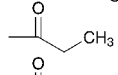


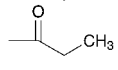
Leucomycin A_1 Acetate: R = 

Leucomycin A_3 Acetate: R = 

Leucomycin A_4 Acetate: R = 

Leucomycin A_5 Acetate: R = 

Leucomycin A_6 Acetate: R = 

Leucomycin A_7 Acetate: R = 

(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3,9-Diacetoxy-5-[4-*O*-acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-2-*O*-acetyl-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Leucomycin A_1 and A_3 Acetates: acyl = 3-methylbutanoyl
Leucomycin A_4 and A_5 Acetates: acyl = butanoyl
Leucomycin A_6 and A_7 Acetates: acyl = propanoyl

[*178234-32-7*, Kitasamycin Acetate]

Kitasamycin Acetate is a derivative of kitasamycin.

It contains not less than 680 μg (potency) and not more than 790 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin Acetate is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.93). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin Acetate occurs as a white to light yellow-white powder.

It is very soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Kitasamycin Acetate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Kitasamycin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water <*2.48*> Not more than 5.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solution—Weigh accurately an amount of Leucomycin A_5 RS equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

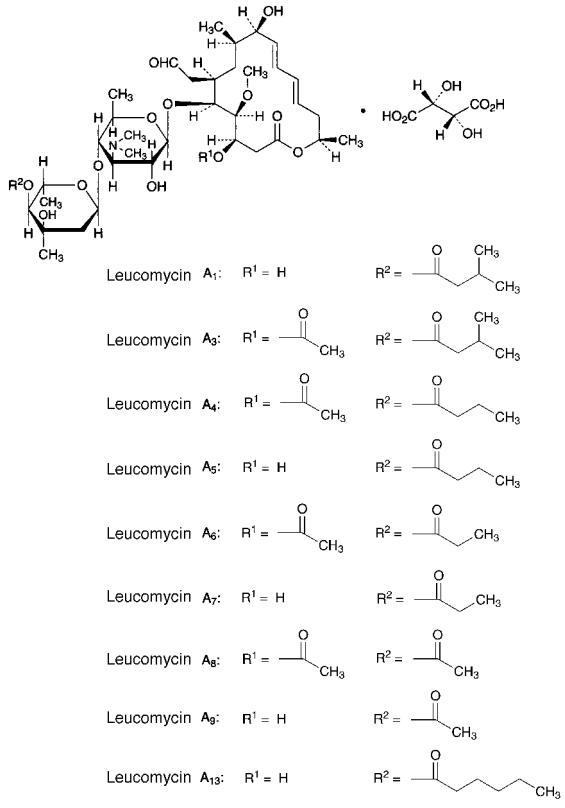
(iv) Sample solution—Weigh accurately an amount of Kitasamycin Acetate equivalent to about 30 mg (potency), dissolve in 25 mL of methanol, add water to make exactly 50 mL, shake well, and allow to stand at 37 ± 2°C for 24 hours. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Kitasamycin Tartrate

Leucomycin Tartrate

キタサマイシン酒石酸塩



(Leucomycin A_1, A_5, A_7, A_9 and A_{13} Tartrates)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate

Leucomycin A_1 Tartrate: acyl = 3-methylbutanoyl
Leucomycin A_5 Tartrate: acyl = butanoyl
Leucomycin A_7 Tartrate: acyl = propanoyl
Leucomycin A_9 Tartrate: acyl = acetyl
Leucomycin A_{13} Tartrate: acyl = hexanoyl

(Leucomycin A_3, A_4, A_6 and A_8 Tartrates)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[4-*O*-acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate

Leucomycin A_3 Tartrate: acyl = 3-methylbutanoyl
Leucomycin A_4 Tartrate: acyl = butanoyl
Leucomycin A_6 Tartrate: acyl = propanoyl
Leucomycin A_8 Tartrate: acyl = acetyl

[*37280-56-1*, Kitasamycin Tartrate]

Kitasamycin Tartrate is the tartrate of kitasamycin. It contains not less than 1300 μg (potency) and not more than 1500 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin Tartrate is expressed as mass (potency) of kitasamycin based on the amount of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.93). One mg (potency) of Kitasamycin Tartrate is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin Tartrate occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Kitasamycin Tartrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Kitasamycin Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 1 g of Kitasamycin Tartrate in 20 mL of water, add 3 mL of sodium hydroxide TS, add 20 mL of *n*-butyl acetate, shake well, and discard the *n*-butyl acetate layer. To the aqueous layer add 20 mL of *n*-butyl acetate, and shake well. The aqueous layer so obtained responds to the Qualitative Tests <*1.09*> (1) for tartrate.

pH <*2.54*> Dissolve 3.0 g of Kitasamycin Tartrate in 100 mL of water: the pH of the solution is between 3.0 and 5.0.

Content ratio of the active principle Dissolve 20 mg of Kitasamycin Tartrate in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 by the area percentage method: the amount of leucomycin A_5 is 40 – 70%, leucomycin A_4 is 5 – 25%, and leucomycin A_1 is 3 – 12%. The relative retention times of leucomycin A_4 and leucomycin A_1 to leucomycin A_5 are about 1.2 and about 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To a suitable amount of a solution of ammonium acetate (77 in 5000) add diluted phosphoric acid (1 in 150) to adjust the pH to 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust so that the retention time of leucomycin A_5 is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin A_5.

System suitability—

System performance: Dissolve about 20 mg each of Leucomycin A_5 RS and Josamycin RS in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A_5 and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A_5 is not more than 1.0%.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Kitasamycin Tartrate in 10 mL of water: the solution is clear and colorless or light yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Kitasamycin Tartrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

Water ⟨*2.48*⟩ Not more than 3.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

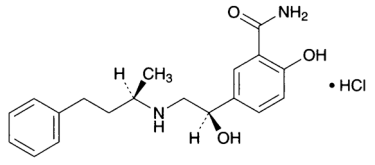
(iii) Standard solutions—Weigh accurately an amount of Leucomycin A_5 RS, equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kitasamycin Tartrate, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

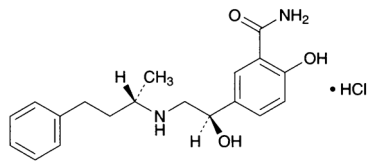
Containers and storage Containers—Tight containers.

Labetalol Hydrochloride

ラベタロール塩酸塩



and enantiomer



and enantiomer

$C_{19}H_{24}N_2O_3$.HCl: 364.87

2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(1*RS*)-1-methyl-3-phenylpropylamino]ethyl}benzamide monohydrochloride

2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(1*SR*)-1-methyl-3-phenylpropylamino]ethyl}benzamide monohydrochloride

[*32780-64-6*]

Labetalol Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl).

Description Labetalol Hydrochloride occurs as a white crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

It dissolves in 0.05 mol/L sulfuric acid TS.

Melting point: about 181°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Labetalol Hydrochloride in 0.05 mol/L sulfuric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Labetalol Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Labetalol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 0.5 g of Labetalol Hydrochloride in 50 mL of water is between 4.0 and 5.0.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Labetalol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.8 g of Labetalol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water, and ammonia solution (28) (25:15:8:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot obtained from the sample solution do not exceed 2 in number and are not more intense than the spot obtained from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Isomer ratio Dissolve 5 mg of Labetalol Hydrochloride in 0.7 mL of a solution of *n*-butylboronic acid in dehydrated pyridine (3 in 250), allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, where A_a is the peak area of the shorter retention time and A_b is the peak area of the longer retention time, using the automatic integration method: the ratio $A_b/(A_a + A_b)$ is between 0.45 and 0.55.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter and 25 m in length, coated inside with methyl silicone polymer for gas chromatography in 5 μm thickness.

Column temperature: A constant temperature of about