ence Standard in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 50 μL each of the sample solution and the 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 Rf value.

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 solution, 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 0.01 g of Estradiol Benzoate Reference Standard, 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.

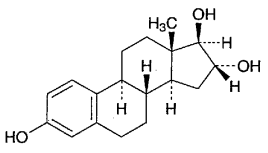
Amount (mg) of estradiol benzoate ($C_{25}H_{28}O_3$)
= amount (mg) of Estradiol Benzoate Reference Standard
$\times \frac{Q_T}{Q_S} \times \frac{1}{5}$

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 $C_{18}H_{24}O_3$.

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 this 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 the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Estriol Reference Standard 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 the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Estriol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +54 – +62° (after drying, 0.04 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point 281 – 286°C

Purity (1) Heavy metals—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) Other steroids—Dissolve 0.040 g 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 the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the 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 Not more than 0.5% (0.5 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.025 g each of Estriol and Estriol Reference Standard, 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 the standard solution as directed under the Liquid Chromatography 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, respectively.

Amount (mg) of $C_{18}H_{24}O_3$
= amount (mg) of Estriol Reference Standard
$\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of methyl benzoate for estriol limit test in methanol (1 in 1000).

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 (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 the flow rate so that the retention time of estriol is about 10 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 estriol and the internal standard in this order with the resolution between these peaks being not less than 8.

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% and not more than 110% 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 according to the labeled amount, 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 the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 279 nm and 283 nm.

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 0.025 g of Estriol Reference Standard, 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.

$$\text{Amount (mg) of estriol } (C_{18}H_{24}O_3) = \text{amount (mg) of Estriol Reference Standard} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—A solution of methyl benzoate for estriol limit 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% and not more than 110% 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 according to the labeled amount, add ethanol (95) to make 20 mL, 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 the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 279 nm and 283 nm.

Content uniformity To one 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 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 test Perform the test with 1 tablet of Estriol Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the test solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 0.1 μg of estriol ($C_{18}H_{24}O_3$) according to the labeled