Carrier gas: Nitrogen

Flow rate: Adjust the flow rate 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 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.

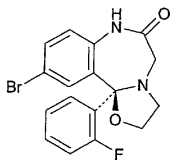
Control solution: Dissolve 0.225 g of thymol for assay in isooctane to make exactly 100 mL. To 10 mL each of this solution, accurately measured, add isooctane to make exactly 150 mL and 100 mL, respectively. Proceed with 0.50 mL each of these solutions in the same manner as Halothane, and use the separated 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

(*RS*)-10-Bromo-11b-(2-fluorophenyl)-2,3,7,11b-tetrahydrooxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one

[*59128-97-1*]

Haloxazolam, when dried, contains not less than 99.0% of $C_{17}H_{14}BrFN_2O_2$.

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 0.01 g 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 0.05 g of Haloxazolam as directed under the Oxygen Flask Combustion Method, 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 for bromide and for fluoride.

(3) Determine the absorption spectrum of a solution of Haloxazolam in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, 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 Haloxazolam, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance $E^{1\%}_{1cm}$ (247 nm): 390 - 410 (0.01 g, 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 the Chloride Limit Test. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS.

(3) Heavy metals—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—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 using Apparatus B: 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 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography 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 the area of the 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 about 4 mm in inside diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: Room temperature.

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 3 volumes of this solution add 2 volumes of acetonitrile.

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

Selection of column: Dissolve 0.01 g each of Haloxazolam and cloxazolam in 200 mL of acetonitrile. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of Haloxazolam and cloxazolam in this order with the resolution between these peaks being not less than 1.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of haloxazolam obtained from 10 μL of the standard solution is between 5 mm and 15 mm.

Time span of measurement: About 3 times as long as the retention time of haloxazolam after the solvent peak.

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

Residue on ignition Not more than 0.10% (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 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
= 37.721 mg of $C_{17}H_{14}BrFN_2O_2$

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

Heparin Sodium

ヘパリンナトリウム

Heparin Sodium is obtained from the livers, the lungs and the intestinal mucosa of healthy edible animals, and prolongs the clotting time of blood. Heparin Sodium obtained from the livers and the lungs contains not less than 110 Heparin Units per mg, and that obtained from the intestinal mucosa contains not less than 130 Heparin Units per mg.

Heparin Sodium, calculated on the dried basis, contains not less than 90% and not more than 110% of the labeled Units.

Label the name of the organ used as the starting material.

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.

pH 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 0.03 g 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 the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not more than 3.0%.

(4) Protein—To 1.0 mL of the sample solution obtained in (2) add 5 drops of a solution of trichloroacetic acid (1 in 5): neither a precipitate nor turbidity is produced.

Loss on drying Not more than 10% (0.02 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 40% (after drying, 0.02 g).

Pyrogen Dissolve Heparin Sodium in isotonic sodium chloride solution so as to contain 1000 Units per mL according to the labeled Units. Inject into rabbits 2 mL of this solution per kg: it meets the requirements of the Pyrogen Test.

Assay (i) Standard solution: Dissolve Heparin Sodium Reference Standard in water so as to contain exactly 2.00 and 1.60 Units per ml, and use them as the high-dose standard solution (S_H) and the low-dose standard solution (S_L), respectively.

(ii) Sample solution: Weigh accurately appropriate amounts of Heparin Sodium according to the labeled Units, dissolve in water so as to contain exactly 2.00 and 1.60 Units per ml, and use them as the high-dose sample solution (T_H) and the low-dose sample solution (T_L), respectively.

(iii) Sulfated whole blood: Place 250 mL of fresh bovine blood in a wide-mouthed stoppered polyethylene bottle containing 50 mL of a solution of sodium sulfate decahydrate (9 in 50), and store at 1 to 4°C. Remove any clotted substance before use.

(iv) Acetone-dried cattle brain: Mince fresh cattle brains after removing blood vessels, connective tissues and other similar matters from them, and place in 10 volumes of acetone to dehydrate. Place 30 g of the dehydrated minced brains in a mortar. Grind with each 75 mL of acetone and dehydrate completely. Dry at 37°C for 2 hours to remove acetone completely.

(v) Thrombokinase extract: To 1.5 g of acetone-dried cattle brain add 60 mL of water, extract at 50°C for 10 to 15 minutes, and centrifuge for 2 minutes at 1500 revolutions per minute. To the supernatant add cresol to make 0.3% as a preservative, and store between 1°C and 4°C. The potency of this solution will be maintained for a few days.