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.
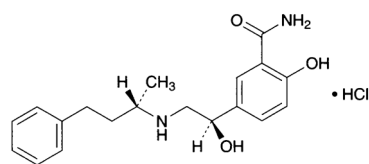
Containers and storage Containers—Tight containers.

Labetalol Hydrochloride

ラベタロール塩酸塩
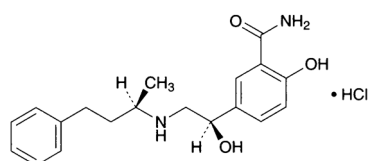
and enantiomer

and enantiomer

$C_{19}H_{24}N_2O_3 \cdot HCl$: 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 ($C_{19}H_{24}N_2O_3 \cdot HCl$).

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

290°C.

Injection port temperature: A constant temperature of about 350°C.

Detector temperature: A constant temperature of about 350°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of the peak showing earlier elution of the two peaks of labetalol is about 9 minutes.

System suitability—

System performance: Proceed with 2 μL of the sample solution under the above conditions: the resolution between the two labetalol peaks is not less than 1.5.

System repeatability: Repeat the test 6 times under the above conditions with 2 μL of the sample solution: the relative standard deviation of the ratio of the peak area of labetalol with the shorter retention time to that of the longer retention time is not more than 2.0%.

Assay Weigh accurately about 0.3 g of Labetalol Hydrochloride, 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 a blank determination in the same manner and make any necessary correction.

<div style="text-align:center">

Each mL of 0.1 mol/L perchloric acid VS
= 36.49 mg of $C_{19}H_{24}N_2O_3.HCl$

</div>

Containers and storage Containers—Tight containers.

Labetalol Hydrochloride Tablets

ラベタロール塩酸塩錠

Labetalol Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$: 364.87).

Method of preparation Prepare as directed under Tablets, with Labetalol Hydrochloride.

Identification **(1)** To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 5 mg of Labetalol Hydrochloride, add 100 mL of 0.05 mol/L sulfuric acid TS, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 300 nm and 304 nm.

(2) To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 0.25 g of Labetalol Hydrochloride, add 25 mL of methanol, shake vigorously for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of labetalol hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test using 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, 2-propanol, water, and ammonia solution (28) (25:15:8:2) 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 the standard solution show the same *R*f value.

Uniformity of dosage units <6.02> Perform the Mass varia-

tion test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Labetalol Hydrochloride Tablets add 5 mL of 0.5 mol/L sulfuric acid TS and 30 mL of water, shake vigorously for 30 minutes, add water to make exactly 50 mL, and filter. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add 0.05 mol/L sulfuric acid TS to make exactly *V* mL so that each mL contains about 40 μg of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS 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 302 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

<div style="text-align:center">

Amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$)
= $M_S \times A_T/A_S \times V/40$

</div>

M_S: Amount (mg) of labetalol 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 Labetalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Labetalol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at 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, and add water to make exactly *V'* mL so that each mL contains about 50 μg of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 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. 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 302 nm.

<div style="text-align:center">

Dissolution rate (%) with respect to the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

</div>

M_S: Amount (mg) of labetalol hydrochloride for assay taken
C: Labeled amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$) in 1 tablet

Assay Weigh accurately not less than 20 Labetalol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$), add 100 mL of 0.5 mol/L sulfuric acid TS and 600 mL of water, shake vigorously for 30 minutes, add water to make exactly 1000 mL, and filter. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, and add 0.05 mol/L sulfuric acid TS to make exactly 25 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 5 mL of this solution,

add 0.05 mol/L sulfuric acid TS 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 Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 302 nm.
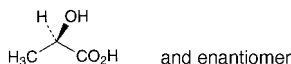
Amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3 \cdot HCl$)
    $= M_S \times A_T/A_S \times 25$

M_S: Amount (mg) of labetalol hydrochloride for assay taken

Containers and storage   Containers—Tight containers.

Lactic Acid

乳酸

$C_3H_6O_3$: 90.08
(2RS)-2-Hydroxypropanoic acid
[50-21-5]

Lactic Acid is a mixture of lactic acid and lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of lactic acid ($C_3H_6O_3$).

Description   Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, unpleasant odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It is hygroscopic.
Specific gravity d^{20}_{20}: about 1.20

Identification   A solution of Lactic Acid (1 in 50) changes blue litmus paper to red and responds to the Qualitative Tests <1.09> for lactate.

Purity   **(1)**   Chloride <1.03>—Perform the test with 1.0 g of Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2)   Sulfate <1.14>—Perform the test with 2.0 g of Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3)   Heavy metals <1.07>—To 2.0 g of Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. 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 from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

(4)   Iron <1.10>—Prepare the test solution with 4.0 g of Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

(5)   Sugars—To 1.0 g of Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling's TS for 5 minutes: no red precipitate is produced.

(6)   Citric, oxalic, phosphoric and L-tartaric acid—To 1.0 g of Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

(7)   Glycerin or mannitol—Shake 10 mL of Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

(8)   Volatile fatty acids—Warm Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

(9)   Cyanide—Transfer 1.0 g of Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) by shaking until a pale red color develops, add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. Cool, add dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, add 10 mL of phosphate buffer solution (pH 6.8), and 0.25 mL of sodium toluensulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make exactly 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

(10)   Readily carbonizable substances—Superimpose slowly 5 mL of Lactic Acid, previously kept at 15°C, upon 5 mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

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

Assay   Weigh accurately about 3 g of Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1 mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
    = 90.08 mg of $C_3H_6O_3$

Containers and storage   Containers—Tight containers.

L-Lactic Acid

L-乳酸
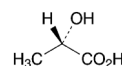
$C_3H_6O_3$: 90.08
(2S)-2-Hydroxypropanoic acid
[79-33-4]

L-Lactic Acid is a mixture of L-lactic acid and L-lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of L-lactic acid ($C_3H_6O_3$).

Description   L-Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, no unpleasant odor.

It is miscible with water, with ethanol (99.5) and with diethyl ether.

It is hygroscopic.
Specific gravity d^{20}_{20}: about 1.20

Identification   A solution of L-Lactic Acid (1 in 50) changes the color of blue litmus paper to red, and responds to the Qualitative Tests <1.09> for lactate.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: $-46 - -52°$ Weigh accurately an amount of L-Lactic Acid, equivalent to about 2 g of L-lactic acid ($C_3H_6O_3$), add exactly 25 mL of 1 mol/L sodium hydroxide VS, cover with a watch glass, and heat on a water bath for 15 minutes. Cool, and adjust to pH 7.0 with 1 mol/L hydrochloric acid VS. Dissolve 5.0 g of hexaammonium heptamolybdate tetrahydrate in this solution, add water to make exactly 50 mL, and determine the optical rotation using a 100-mm cell.

Purity **(1)** Chloride ⟨*1.03*⟩—Perform the test with 1.0 g of L-Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate ⟨*1.14*⟩—Perform the test with 2.0 g of L-Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals ⟨*1.07*⟩—To 2.0 g of L-Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. 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 from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

(4) Iron ⟨*1.10*⟩—Prepare the test solution with 4.0 g of L-Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Sugars—To 1.0 g of L-Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling's TS for 5 minutes: no red precipitate is produced.

(6) Citric, oxalic, phosphoric and L-tartaric acid—To 1.0 g of L-Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

(7) Glycerin or mannitol—Shake 10 mL of L-Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

(8) Volatile fatty acids—Warm L-Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

(9) Cyanide—Transfer 1.0 g of L-Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) while shaking until a pale red color develops, then add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. After cooling, add dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, 10 mL of phosphate buffer solution (pH 6.8) and 0.25 mL of sodium toluenesulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

(10) Readily carbonizable substances—Superimpose slowly 5 mL of L-Lactic Acid, previously kept at 15°C, upon 5 mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

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

Assay Weigh accurately about 3 g of L-Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1

mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

<div align="center">

Each mL of 1 mol/L sodium hydroxide VS
= 90.08 mg of $C_3H_6O_3$

</div>

Containers and storage Containers—Tight containers.

Anhydrous Lactose

無水乳糖
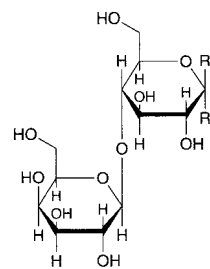
α-Lactose : R[1]=H, R[2]=OH
β-Lactose : R[1]=OH, R[2]=H

$C_{12}H_{22}O_{11}$: 342.30
β-D-Galactopyranosyl-(1→4)-β-D-glucopyranose
(β-lactose)
β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose
(α-lactose)
[63-42-3, Anhydrous Lactose]

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 Lactose is β-lactose or a mixture of β-lactose and α-lactose.
◆The relative quantities of α-lactose and β-lactose in Anhydrous Lactose is labeled as the isomer ratio.◆

◆**Description** Anhydrous Lactose occurs as white, crystals or powder.
It is freely soluble in water, and practically insoluble in ethanol (99.5).◆

Identification Determine the infrared absorption spectrum of Anhydrous Lactose, 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 Anhydrous Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: $+54.4 - +55.9°$ Weigh accurately about 10 g of Anhydrous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of boiling water, and allow to cool: the solution is clear, and colorless or nearly colorless and has no more color than the following control solution. Determine the absorbance at 400 nm of this solution as di-

rected under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.04.

Control solution: To a mixture of 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS, add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pink or red color.

♦**(3)** Heavy metals <1.07>—Proceed with 4.0 g of Anhydrous Lactose according to Method 2, and perform the test. Prepare the control solution with 2 mL of Standard Lead Solution (not more than 5 ppm).♦

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 2 hours).

Water <2.48> Not more than 1.0% (1 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% (1 g).

Microbial limit <4.05> The acceptance criteria of TAMC is 10^2 CFU/g, ♦and that of TYMC is 5×10^1 CFU/g,♦ and ♦*Salmonella* and♦ *Escherichia coli* are not observed.

Isomer ratio Place 10 mg of Anhydrous Lactose in a screw capped reaction vial for gas chromatography, add 4 mL of a mixture of pyridine, trimethylsilylimidazole and dimethylsulfoxide (117:44:39), stopper, and exposure to ultrasonic waves at room temperature for 20 minutes. After cooling, transfer 400 μL of this solution into a vial for injection, add 1 mL of pyridine, stopper tightly, mix, and use this fluid as the sample solution. Perform the test with 0.5 μL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the peak areas of α-lactose and β-lactose, A_a and A_b, and calculate the contents (%) of α-lactose and β-lactose in Anhydrous Lactose by the following equations.

$$\text{Content (\%) of } \alpha\text{-lactose} = A_a/(A_a + A_b) \times 100$$

$$\text{Content (\%) of } \beta\text{-lactose} = A_b/(A_a + A_b) \times 100$$

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 15 m in length, coated the inside surface with 5% diphenyl-95% dimethylpolysiloxane in 0.25 μm thickness. Use a middle polar inertness fused silica column 0.53 mm in inside diameter and 2 m in length as a guard column.

Column temperature: Keep at 80°C for 1 minute after injection, then rise to 150°C with 35°C per minute, then rise to 300°C with 12°C per minute, and keep 300°C for 2 minutes.

Injection port temperature: A constant temperature of about 275°C, or use cold-on column injection.

Detector temperature: A constant temperature of about 325°C.

Carrier gas: Helium.

Flow rate: 2.8 mL per minute (Retention time of β-lactose is about 12 minutes).

Sprit ratio: Spritless.

System suitability—

System performance: Prepare a solution with 10 mg of a mixture of α-lactose and β-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 0.5 μL of this solution under the above operating conditions, and determine the retention times of the peaks of α-lactose and β-lactose: the relative retention time of α-lactose to that of β-lactose is about 0.9 with the resolution between these peaks being not less than 3.0.
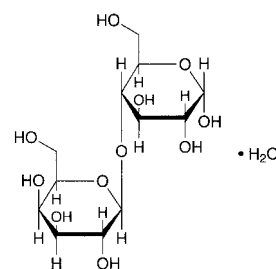
♦System repeatability: When the test is repeated 6 times with 0.5 μL of the solution used in the system performance under the above operating conditions, the relative standard deviation of the peak area of β-lactose is not more than 1.0%.♦

♦**Containers and storage** Containers—Well-closed containers.♦

Lactose Hydrate

Lactose

乳糖水和物

$C_{12}H_{22}O_{11}.H_2O$: 360.31
β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate
[*64044-51-5*, Mixture of α- and β-lactose monohydrate]

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 (♦   ♦).

Lactose Hydrate is the monohydrate of β-D-galactopyranosyl-(1→4)-α-D-glucopyranose.
♦It is a disaccharide obtained from milk, consist of one unit of glucose and one unit of galactose.♦
♦The label states the effect where it is the granulated powder.♦

♦**Description** Lactose Hydrate occurs as white, crystals, powder or granulated powder.

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

Identification Determine the infrared absorption spectrum of Lactose Hydrate, 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 Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +54.4 − +55.9°. Weigh accurately about 10 g of Lactose Hydrate, calculated on the

anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Lactose Hydrate in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Lactose Hydrate by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

◆**(3)** Heavy metals <1.07>—Dissolve 4.0 g of Lactose Hydrate in 20 mL of warm water, add 1 mL of 0.1 mol/L hydrochloric 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 1 mL of 0.1 mol/L hydrochloric acid TS and 2.0 mL of Standard Lead Solution (not more than 5 ppm).◆

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Lactose Hydrate in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

◆**Loss on drying** <2.41> Not more than 0.5%. For the granulated powder, not more than 1.0% (1 g, 80°C, 2 hours).◆

Water <2.48> 4.5 – 5.5%. ◆For the granulated powder, 4.0 – 5.5%◆ (1 g, volumetric titration, direct titration. Use a mixture of methanol for water determination and formamide for water determination (2:1) instead of methanol for water determination).
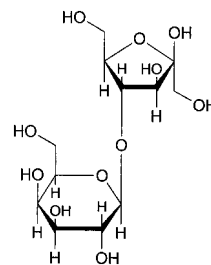
Residue on ignition <2.44> Not more than 0.1% (1 g).

◆**Microbial limit** <4.05> The acceptance criteria of TAMC and TYMC are 10^2 CFU/g and 5×10^1 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.◆

◆**Containers and storage** Containers—Well-closed containers.◆

Lactulose

ラクツロース

$C_{12}H_{22}O_{11}$: 342.30
β-D-Galactopyranosyl-(1→4)-D-fructose
[*4618-18-2*]

Lactulose is a solution of lactulose prepared by isomerizing lactose under the existing of alkaline and purified by ion-exchange resin.

It contains not less than 50.0% and not more than 56.0% of lactulose ($C_{12}H_{22}O_{11}$).

Description Lactulose occurs as a clear, colorless or light yellow, viscous liquid. It is odorless, and has a sweet taste.

It is miscible with water and with formamide.

Identification **(1)** To 0.7 g of Lactulose add 10 mL of water, 10 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 25) and 0.2 mL of acetic acid (100), and heat in a water bath for 5 to 10 minutes: a blue color develops.

(2) Mix 0.3 g of Lactulose and 30 mL of water, add 16 mL of 0.5 mol/L iodine TS, then immediately add 2.5 mL of 8 mol/L sodium hydroxide TS, allow to stand for 7 minutes, and add 2.5 mL of diluted sulfuric acid (3 in 20). To this solution add a saturated solution of sodium sulfite heptahydrate until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25), and add water to make 100 mL. To 10 mL of this solution add 5 mL of Fehling's TS, and boil for 5 minutes: a red precipitate is produced.

pH <2.54> To 2.0 g of Lactulose add 15 mL of water: the pH of the solution is between 3.5 and 5.5.

Specific gravity <2.56> d_{20}^{20}: 1.320 – 1.360

Purity **(1)** Heavy metals <1.07>—Proceed with 5.0 g of Lactulose according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Lactulose according to Method 1, and perform the test (not more than 2 ppm).

(3) Galactose and lactose—Determine the heights of the peaks corresponding to galactose and lactose respectively, on the chromatogram obtained in Assay from the sample solution and the standard solution, and calculate the ratios of the peak heights of galactose and lactose to that of the internal standard from the sample solution, Q_{Ta} and Q_{Tb}, and then from the standard solution, Q_{Sa} and Q_{Sb}: it contains galactose of not more than 11%, and lactose of not more than 6%.

Amount (mg) of galactose ($C_6H_{12}O_6$)
$$= M_S \times Q_{Ta}/Q_{Sa}$$

M_S: Amount (mg) of galactose taken

$$\text{Amount (mg) of lactose } (C_{12}H_{22}O_{11}.H_2O)$$
$$= M_S \times Q_{Tb}/Q_{Sb}$$

M_S: Amount (mg) of lactose Hydrate taken

Loss on drying <2.41> Not more than 35% (0.5 g, in vacuum, 80°C, 5 hours).

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

Assay Weigh accurately about 1 g of Lactulose, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of Lactulose RS, about 80 mg of D-galactose and about 40 mg of lactose monohydrate, add exactly 10 mL of the internal standard solution and water 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 height of lactulose to that of the internal standard.

$$\text{Amount (mg) of lactulose } (C_{12}H_{22}O_{11})$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Lactulose RS taken

Internal standard solution—A solution of D-mannitol (1 in 20).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column 8 mm in inside diameter and 50 cm in length, packed with gel type strongly acidic ion-exchange resin for liquid chromatography (degree of crosslinkage: 6%) (11 μm in particle diameter).

Column temperature: A constant temperature of about 75°C.

Mobile phase: Water.

Flow rate: Adjust so that the retention time of lactulose is about 18 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lactulose 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 heights of lactulose, galactose and lactose to the height of the internal standard are not more than 2.0%, respectively.

Containers and storage Containers—Tight containers.

Lafutidine

ラフチジン

and enantiomer
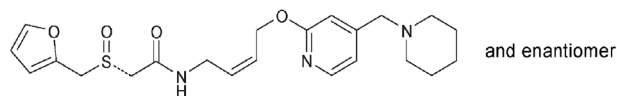
$C_{22}H_{29}N_3O_4S$: 431.55

2-[(*RS*)-Furan-2-ylmethylsulfinyl]-*N*-{4-[4-(piperidin-1-ylmethyl)pyridin-2-yl]oxy-(2*Z*)-but-2-en-1-yl}acetamide
[*206449-93-6*]

Lafutidine, when dried, contains not less than 99.0% and not more than 101.0% of lafutidine ($C_{22}H_{29}N_3O_4S$).

Description Lafutidine occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Lafutidine in methanol (1 in 100) shows no optical rotation.

Lafutidine shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Lafutidine 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.

(2) Determine the infrared absorption spectrum of Lafutidine, 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>—Proceed with 2.0 g of Lafutidine 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 0.10 g of Lafutidine 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 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 by the automatic integration method: the area of the peak, having the relative retention time of about 0.85 to lafutidine, from the sample solution is not larger than 3/10 times the peak area of lafutidine from the standard solution, the area of the peak other than lafutidine and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of lafutidine from the standard solution, and the total area of the peaks other than lafutidine from the sample solution is not larger than 2/5 times the peak area of lafutidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of diluted phosphoric acid (1 in 1000). To 850 mL of this solution add 150 mL of acetonitrile.

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

Time span of measurement: About 6 times as long as the retention time of lafutidine.

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 lafutidine 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, the number of theoretical plates and the symmetry factor of the peak of lafutidine are not less than 8000 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 lafutidine 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, phosphorus (V) oxide, 4 hours).

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

Assay   Weigh accurately about 0.3 g of Lafutidine, 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
= 21.58 mg of $C_{22}H_{29}N_3O_4S$

Containers and storage   Containers—Tight containers.

Lafutidine Tablets

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Lafutidine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lafutidine ($C_{22}H_{29}N_3O_4S$: 431.55).

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

Identification   Powder Lafutidine Tablets. To a portion of the powder, equivalent to 10 mg of Lafutidine, add 10 mL of methanol, shake thoroughly, 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 an absorption maximum between 271 nm and 275 nm.

Purity   Related substances—To 10 Lafutidine Tablets add $4V/5$ mL of the mobile phase, disintegrate the tablets with the aid of ultrasonic waves, then shake vigorously for not less than 30 minutes, and add the mobile phase to make V mL so that each mL contains about 1 mg of lafutidine ($C_{22}H_{29}N_3O_4S$). Centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solu-

tion 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 by the automatic integration method: the area of the peak, other than lafutidine and the peak having the relative retention time of about 0.85 to lafutidine, from the sample solution is not larger than 1/5 times the peak area of lafutidine from the standard solution, and the total area of the peaks, other than lafutidine and the peak having the relative retention time of about 0.85 to lafutidine, from the sample solution is not larger than 3/5 times the peak area of lafutidine 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 6 times as long as the retention time of lafutidine.

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 lafutidine 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, the number of theoretical plates and the symmetry factor of the peak of lafutidine are not less than 8000 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 lafutidine 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 Lafutidine Tablets add exactly V mL of the internal standard solution so that each mL contains about 2 mg of lafutidine ($C_{22}H_{29}N_3O_4S$), disintegrate the tablet with the aid of ultrasonic waves, then shake vigorously for 30 minutes. Centrifuge this solution, filter the supernatant liquid 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 0.1 g of lafutidine for assay, previously dried under a reduced pressure (not exceeding 0.67 kPa) using phosphorus (V) oxide as dessicant for 4 hours, dissolve in exactly 50 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of lafutidine ($C_{22}H_{29}N_3O_4S$)
= $M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of lafutidine for assay taken

Internal standard solution—A solution of ethyl aminobenzoate in a mixture of acetonitrile and water (4:1) (3 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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Lafutidine Tablets is not less than 75%.

Start the test with 1 tablet of Lafutidine 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 lafutidine ($C_{22}H_{29}N_3O_4S$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of lafutidine for assay, previously dried under a reduced pressure (not exceeding 0.67 kPa) using phosphorus (V) oxide as dessicant for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 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 25 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 lafutidine in each solution.

Dissolution rate (%) with respect to the labeled amount of lafutidine ($C_{22}H_{29}N_3O_4S$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of lafutidine for assay taken
C: Labeled amount (mg) of lafutidine ($C_{22}H_{29}N_3O_4S$) 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 25 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of lafutidine are not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lafutidine is not more than 2.0%.

Assay To 20 Lafutidine Tablets add $4V/5$ mL of the internal standard solution, disintegrate the tablets with the aid of ultrasonic waves, then shake vigorously for 30 minutes. Add the internal standard solution to make exactly V mL so that each mL contains about 2 mg of lafutidine ($C_{22}H_{29}N_3O_4S$), centrifuge, filter the supernatant liquid 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 0.1 g of lafutidine for assay, previously dried under a reduced pressure (not exceeding 0.67 kPa) using phosphorus (V) oxide as dessicant for 4 hours, dissolve in the internal standard solution to make exactly 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 lafutidine to that of the internal standard.

Amount (mg) of lafutidine ($C_{22}H_{29}N_3O_4S$) in 1 tablet
$$= M_S \times Q_T/Q_S \times V/1000$$

M_S: Amount (mg) of lafutidine for assay taken

*Internal standard solution—*A solution of ethyl aminobenzoate in a mixture of acetonitrile and water (4:1) (3 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 275 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: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of diluted phosphoric acid (1 in 1000). To 850 mL of this solution add 150 mL of acetonitrile.
Flow rate: Adjust so that the retention time of lafutidine 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, lafutidine 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 5 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of lafutidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Lanatoside C

ラナトシド C
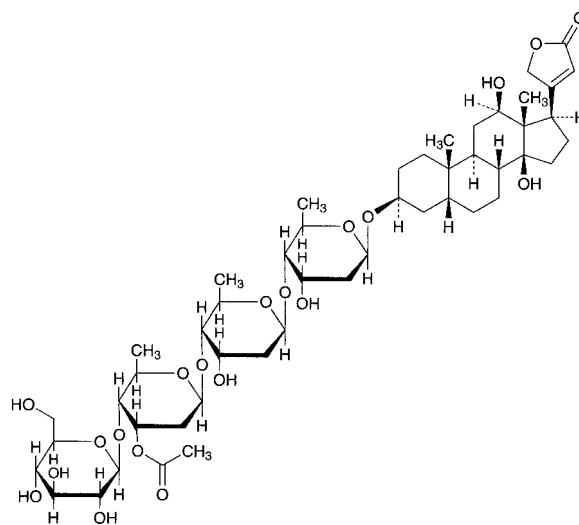
$C_{49}H_{76}O_{20}$: 985.12
3β-[β-D-Glucopyranosyl-(1→4)-3-O-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide
[*17575-22-3*]

Lanatoside C, when dried, contains not less than 90.0% and not more than 102.0% of lanatoside C ($C_{49}H_{76}O_{20}$).

Description Lanatoside C occurs as colorless or white crystals or a white crystalline powder. It is odorless.
It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.
It is hygroscopic.

Identification Place 1 mg of Lanatoside C to a small test tube having an internal diameter of about 10 mm, 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 is produced, and the color of the upper layer near the contact zone gradually changes to blue through purple. Finally the color of the entire acetic acid layer changes to blue-green through deep blue.

Purity Related substances—Dissolve 10 mg of Lanatoside C in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of Lanatoside C RS in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03> with these solutions. 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: any spots other than the principal spot from the sample solution are neither larger nor darker than the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+32 - +35°$ (after drying, 0.5 g, methanol, 25 mL, 100 mm).

Loss on drying <2.41> Not more than 7.5% (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 Weigh accurately about 50 mg each of Lanatoside C and Lanatoside C RS, previously dried, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution into 25-mL light-resistant, volumetric flasks, and add 5 mL of 2,4,6-trinitrophenol TS and 0.5 mL of a solution of sodium hydroxide (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, A_T and A_S, of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

Amount (mg) of lanatoside C ($C_{49}H_{76}O_{20}$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Lanatoside C RS taken

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

Lanatoside C Tablets

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Lanatoside C Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$: 985.12).

Method of preparation Prepare as directed under Tablets, with Lanatoside C.

Identification (1) Shake a quantity of powdered Lanatoside C Tablets, equivalent to 1 mg of Lanatoside C, with 3 mL of diethyl ether, and filter. Wash the residue with two 3-mL portions of diethyl ether, and air-dry. To the remaining residue add 10 mL of a mixture of chloroform and methanol (9:1), shake, and filter. Wash the residue with two 5-mL portions of a mixture of chloroform and methanol (9:1), combine the filtrate and washings, and evaporate on a water

bath to a smaller volume. Transfer the solution to a small test tube having an internal diameter of about 10 mm, further evaporate on a water bath to dryness, and proceed as directed in the Identification under Lanatoside C.

(2) Perform the test with the sample solution and the standard solution obtained in the Assay as directed under Thin-layer Chromatography <2.03>. Spot 25 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 on the plate, and heat the plate at 110°C for 10 minutes: the spots obtained from the sample solution and standard solution show a black color, and have the same Rf values.

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

Warm 1 tablet of Lanatoside C Tablets with 5 mL of water until the tablet is disintegrated, add 30 mL of ethanol (95), disperse finely the particles with the aid of ultrasonic waves, add ethanol (95) to make exactly V mL of a solution containing about 5 μg of lanatoside C ($C_{49}H_{76}O_{20}$) in each 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 of Lanatoside C RS, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 2 mL of this solution, add 10 mL of water, add ethanol (95) 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) (17 in 20) into three brown glass-stoppered test tubes T, S and B, previously containing exactly 10 mL of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, add exactly 1 mL each of dilute hydrogen peroxide TS immediately, shake vigorously, and allow to stand at a constant temperature between 25°C and 30°C for 40 minutes. Determine the fluorescence intensities, F_T, F_S and F_B, of the subsequent solutions from the sample solution, the standard solution and the diluted ethanol (95) (17 in 20) at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

Amount (mg) of lanatoside C ($C_{49}H_{76}O_{20}$)
$= M_S \times (F_T - F_B)/(F_S - F_B) \times V/5000$

M_S: Amount (mg) of Lanatoside C RS taken

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

Start the test with 1 tablet of Lanatoside C 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 contains about 0.5 μg of lanatoside ($C_{49}H_{76}O_{20}$), and use this solution as the sample solution. Separately, dry Lanatoside C RS in vacuum over phosphorus (V) oxide at 60°C for 4 hours, weigh accurately a portion of it, equivalent to 100 times an amount of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$), dissolve in ethanol (95) to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 500 mL, warm at 37 ± 0.5°C for 60

minutes, and use this solution as the standard solution. Pipet 3 mL each of the sample solution, the standard solution and the dissolution medium, and transfer to glass-stoppered brown test tubes T, S and B, respectively. To these solutions add exactly 10 mL each of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, and shake. Immediately add exactly 0.2 mL each of diluted hydrogen peroxide TS (1 in 100), shake well, and allow to stand at a constant temperature between 30°C and 37°C for 45 minutes. Determine immediately the fluorescence intensities, F_T, F_S and F_B, of the sample solution, the standard solution and the dissolution medium at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>.

Dissolution rate (%) with respect to the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$)

$$= M_S \times (F_T - F_B)/(F_S - F_B) \times V'/V \times 1/C$$

M_S: Amount (mg) of Lanatoside C RS taken

C: Labeled amount (mg) of lanatoside C ($C_{49}H_{76}O_{20}$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Lanatoside C Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of lanatoside C ($C_{45}H_{76}O_{20}$), into a 100-mL light-resistant volumetric flask, add 50 mL of ethanol (95), and shake for 15 minutes. Then dilute with ethanol (95) to make 100 mL. Filter this solution, discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 5 mg of Lanatoside C RS, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, dissolve in ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution into light-resistant, glass-stoppered test tubes, add exactly 3 mL each of alkaline 2,4,6-trinitrophenol TS, shake well and allow these solutions to stand between 22°C and 28°C for 25 minutes. Determine the absorbances, A_T and A_S, of the subsequent sample solution and the subsequent standard solution at 490 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by the same manner with 5 mL of ethanol (95), as the blank.

Amount (mg) of lanatoside C ($C_{49}H_{76}O_{20}$)
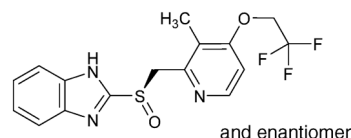
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of Lanatoside C RS taken

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

Lansoprazole

ランソプラゾール

and enantiomer

$C_{16}H_{14}F_3N_3O_2S$: 369.36
(RS)-2-({[3-Methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methyl}sulfinyl)-1H-benzimidazole
[*103577-45-3*]

Lansoprazole contains not less than 99.0% and not more than 101.0% of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$), calculated on the anhydrous basis.

Description Lansoprazole occurs as a white to brownish white crystalline powder.

It is freely soluble in N, N-dimethylformamide, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Lansoprazole in N, N-dimethylformamide (1 in 10) shows no optical rotation.

Melting point: about 166°C (with decomposition).

It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Lansoprazole 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 Lansoprazole 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 Lansoprazole 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 Lansoprazole RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Lansoprazole in 20 mL of N, N-dimethylformamide: the solution is clear and not more colored than Matching Fluid G.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Lansoprazole in a platinum crucible 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) Arsenic <1.11>—Prepare the test solution with 1.0 g of Lansoprazole in a platinum crucible according to Method 3, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), and perform the test. Prepare the standard color with 1.0 mL of Standard Arsenic Solution (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Lansoprazole in a mixture of dilute sodium hydroxide TS and methanol (3:1) to make 20 mL. To 2 mL of this solution add a mixture of dilute sodium hydroxide TS and methanol (3:1) to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of dilute sodium hydroxide TS and methanol (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 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 1.1 to lansoprazole, obtained from the sample solution is not larger than 2/5 times the peak area of lansoprazole from the standard solution, and the area of the peak other than lansoprazole and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of lansoprazole from the standard solution. Furthermore, the total area of the peaks other than lansoprazole from the sample solution is not larger than 3/5 times the peak area of lansoprazole from the standard solution. For the area of the peaks, having the relative retention time of about 0.8, about 1.1 and about 1.2 to lansoprazole, multiply their relative response factors, 0.8, 1.2, and 1.3, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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: Water.

Mobile phase B: A mixture of acetonitrile, water and triethylamine (160:40:1), adjusted to pH 7.0 with phosphoric acid.

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 – 40	90 → 20	10 → 80
40 – 50	20	80

Flow rate: About 0.8 mL per minute (the retention time of lansoprazole is about 29 minutes).

Time span of measurement: About 1.7 times as long as the retention time of lansoprazole.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of dilute sodium hydroxide TS and methanol (3:1) to make exactly 20 mL. Confirm that the peak area of lansoprazole obtained with 40 μL of this solution is equivalent to 4% to 6% of that with 40 μL of the standard solution.

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of lansoprazole are not less than 150,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lansoprazole is not more than 3.0%.

Water *<2.48>*   Not more than 0.10% (0.5 g, coulometric titration).

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

Assay   Weigh accurately about 50 mg each of Lansoprazole and Lansoprazole RS (separately determine the water *<2.48>* in the same manner as Lansoprazole), and dissolve each in exactly 10 mL of the internal standard solution. To 1 mL each of both solutions add diluting solution to make 50 mL, and use these solutions as the sample solution and the stand-

ard 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 lansoprazole to that of the internal standard.

$$\text{Amount (mg) of lansoprazole } (C_{16}H_{14}F_3N_3O_2S)$$
$$= M_S \times Q_T/Q_S$$

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

Internal standard solution—A solution of 4′-ethoxyacetophenone in diluting solution (1 in 400).

Diluting solution: A mixture of water, acetonitrile and triethylamine (60:40:1), adjusted to pH 11.0 with phosphoric acid.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silicon polymer coated 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 (60:40:1), adjusted to pH 7.0 with phosphoric acid.

Flow rate: Adjust so that the retention time of lansoprazole 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, lansoprazole 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 lansoprazole to that of the internal standard is not more than 1.0%.

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

Lansoprazole Delayed-release Capsules

ランソプラゾール腸溶カプセル

Lansoprazole Delayed-release Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$: 369.36).

Method of preparation   Prepare as directed under Capsules, with Lansoprazole.

Identification   Take out the contents of Lansoprazole Delayed-release Capsules, and powder. To a portion of the powder, equivalent to 5 mg of Lansoprazole, add 5 mL of methanol, shake thoroughly, and centrifuge. To 0.1 mL of the supernatant liquid add 10 mL of methanol, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 282 nm and 286 nm.

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 contents of 1 capsule of Lansoprazole Delayed-release Capsules, add $3V/10$ mL of dilute sodium hydroxide TS, and sonicate with occasional stirring to disintegrate the contents completely. Add acetonitrile to make exactly V mL so that each mL contains about 0.15 mg of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$). Centrifuge this solution, filter the supernatant liquid 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 a mixture of acetonitrile and dilute sodium hydroxide TS (7:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Lansoprazole RS (separately determine the water *<2.48>* in the same manner as Lansoprazole), and dissolve in 60 mL of dilute sodium hydroxide TS, and add acetonitrile to make exactly 200 mL. Pipet 4 mL of this solution, add a mixture of acetonitrile and dilute sodium hydroxide TS (7:3) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 294 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

$$\text{Amount (mg) of lansoprazole (}C_{16}H_{14}F_3N_3O_2S\text{)}$$
$$= M_S \times A_T/A_S \times V/200$$

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

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

Assay   Take out the contents of not less than 20 capsules of Lansoprazole Delayed-release Capsules. Weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.3 g of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$), add 60 mL of dilute sodium hydroxide TS, sonicate, and shake thoroughly. To this solution add 20 mL of acetonitrile and exactly 20 mL of the internal standard solution, shake thoroughly, and centrifuge. To 1 mL of the supernatant liquid add diluting solution to make 30 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 mg of Lansoprazole RS (separately determine the water *<2.48>* in the same manner as Lansoprazole), dissolve in 6 mL of dilute sodium hydroxide TS and 2 mL of acetonitrile, and add exactly 2 mL of the internal standard solution. To 1 mL of this solution add diluting solution to make 30 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 lansoprazole to that of the internal standard.

$$\text{Amount (mg) of lansoprazole (}C_{16}H_{14}F_3N_3O_2S\text{)}$$
$$= M_S \times Q_T/Q_S \times 10$$

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

Internal standard solution—A solution of 4′-ethoxyacetophenone in acetonitrile (3 in 400).

Diluting solution: A mixture of water, acetonitrile and triethylamine (60:40:1), adjusted to pH 11.0 with phosphoric acid.

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Lansoprazole.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lansoprazole 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 lansoprazole to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Lansoprazole Delayed-release Orally Disintegrating Tablets

ランソプラゾール腸溶性口腔内崩壊錠

Lansoprazole Delayed-release Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$: 369.36).

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

Identification   Powder 10 tablets of Lansoprazole Delayed-release Orally Disintegrating Tablets. To a portion of the powder, equivalent to 5 mg of Lansoprazole, add 5 mL of methanol, shake thoroughly, and centrifuge. To 0.1 mL of the supernatant liquid add 10 mL of methanol, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 282 nm and 286 nm.

Purity   Related substances—Keep the sample solution and standard solution at not exceeding 5°C, and use them within 12 hours. Powder not less than 10 tablets of Lansoprazole Delayed-release Orally Disintegrating Tablets. To a portion of the powder, equivalent to 25 mg of Lansoprazole, add 10 mL of a mixture of dilute sodium hydroxide TS and methanol (3:1), treat with ultrasonic waves, shake thoroughly, and centrifuge. To 2 mL of the supernatant liquid add diluting solution to make 20 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add diluting solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 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 1.1 to lansoprazole, obtained from the sample solution is not larger than 2/5 times the peak area of lansoprazole from the standard solution, and the area of the peak other than lansoprazole and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of lansoprazole from the standard solution. Furthermore, the total area of the peaks other than lansoprazole from the sample solution is not larger than 1.6 times the peak area of lansoprazole from the standard solution.

Diluting solution: A mixture of acetonitrile, water and

triethylamine (160:40:1), adjusted to pH 11.0 with phosphoric acid. To 100 mL of this solution add 900 mL of water.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, and time span of measurement: Proceed as directed in the operating conditions in the Purity (4) under Lansoprazole.

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 – 30	90 → 20	10 → 80
30 – 40	20	80

Flow rate: About 0.8 mL per minute (the retention time of lansoprazole is about 24 minutes).

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add diluting solution to make exactly 20 mL. Confirm that the peak area of lansoprazole obtained with 40 μL of this solution is equivalent to 4 to 6% of that with 40 μL of the standard solution.

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of lansoprazole are not less than 150,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lansoprazole is not more than 3.0%.

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 Lansoprazole Delayed-release Orally Disintegrating Tablets add 3V/10 mL of dilute sodium hydroxide TS, and sonicate with occasional stirring to disintegrate the tablet completely. Add acetonitrile to make exactly V mL so that each mL contains about 0.15 mg of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$). Centrifuge this solution, and filter the supernatant liquid 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 a mixture of acetonitrile and dilute sodium hydroxide TS (7:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Lansoprazole RS (separately determine the water <2.48> in the same manner as Lansoprazole), and dissolve in 60 mL of dilute sodium hydroxide TS, and add acetonitrile to make exactly 200 mL. Pipet 4 mL of this solution, add a mixture of acetonitrile and dilute sodium hydroxide TS (7:3) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 294 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of lansoprazole } (C_{16}H_{14}F_3N_3O_2S)$$
$$= M_S \times A_T/A_S \times V/200$$

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

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

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

Assay Weigh accurately the mass of not less than 20 tablets of Lansoprazole Delayed-release Orally Disintegrating Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.3 g of lansoprazole ($C_{16}H_{14}F_3N_3O_2S$), add 60 mL of dilute sodium hydroxide TS, sonicate, and shake thoroughly. To this solution add 20 mL of acetonitrile and exactly 20 mL of the internal standard solution, shake thoroughly, and centrifuge. To 1 mL of the supernatant liquid add diluting solution to make 30 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 mg of Lansoprazole RS (separately determine the water <2.48> in the same manner as Lansoprazole), dissolve in 6 mL of dilute sodium hydroxide TS and 2 mL of acetonitrile, and add exactly 2 mL of the internal standard solution. To 1 mL of this solution add diluting solution to make 30 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 lansoprazole to that of the internal standard.

$$\text{Amount (mg) of lansoprazole } (C_{16}H_{14}F_3N_3O_2S)$$
$$= M_S \times Q_T/Q_S \times 10$$

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

Internal standard solution—A solution of 4′-ethoxyacetophenone in acetonitrile (3 in 400).

Diluting solution: A mixture of water, acetonitrile and triethylamine (60:40:1), adjusted to pH 11.0 with phosphoric acid.

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Lansoprazole.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lansoprazole 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 lansoprazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Latamoxef Sodium

ラタモキセフナトリウム
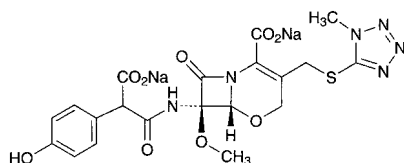
C$_{20}$H$_{18}$N$_6$Na$_2$O$_9$S: 564.44
Disodium (6*R*,7*R*)-7-[2-carboxylato-
2-(4-hydroxyphenyl)acetylamino]-7-methoxy-3-(1-methyl-
1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-oxa-
1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*64953-12-4*]

　Latamoxef Sodium contains not less than 830 μg
(potency) and not more than 940 μg (potency) per mg,
calculated on the anhydrous basis. The potency of
Latamoxef Sodium is expressed as mass (potency) of
latamoxef (C$_{20}$H$_{20}$N$_6$O$_9$S: 520.47).

Description　Latamoxef Sodium occurs as white to light yel-
lowish white, powder or masses.
　It is very soluble in water, freely soluble in methanol, and
slightly soluble in ethanol (95).

Identification　**(1)**　Determine the absorption spectrum of a
solution of Latamoxef 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.
　(2)　Determine the infrared absorption spectrum of
Latamoxef 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)　Determine the ^{1}H spectrum of a solution of Latamox-
ef Sodium in heavy water for nuclear magnetic resonance
spectroscopy (1 in 10) as directed under Nuclear Magnetic
Resonance Spectroscopy <*2.21*>, using sodium 3-trimethyl-
silylpropanesulfonate for nuclear magnetic resonance spec-
troscopy as an internal reference compound: it exhibits
single signals, A and B, at around δ 3.5 ppm and at around
δ 4.0 ppm. The ratio of the integrated intensity of these sig-
nals, A:B, is about 1:1.
　(4)　Latamoxef Sodium responds to the Qualitative Tests
<*1.09*> (1) for sodium salt.

Optical rotation <*2.49*>　$[\alpha]_D^{20}$: $-32 \sim -40°$ (0.5 g calculated
on the anhydrous basis, phosphate buffer solution (pH 7.0),
50 mL, 100 mm).

pH <*2.54*>　The pH of a solution obtained by dissolving
1.0 g of Latamoxef Sodium in 10 mL of water is between 5.0
and 7.0.

Purity　**(1)**　Clarity and color of solution—Dissolve 1.0 g
of Latamoxef Sodium in 10 mL of water: the solution is
clear and has no more color than the following control solu-
tion.
　Control solution: To a mixture of 3.0 mL of Cobalt (II)
Chloride CS and 36 mL of Iron (III) Chloride CS add 11 mL
of diluted dilute hydrochloric acid (1 in 10). To 2.5 mL of
this solution add 7.5 mL of diluted dilute hydrochloric acid

(1:10).
　(2)　Heavy metals <*1.07*>—Carbonize 1.0 g of Latamoxef
Sodium by heating gently, previously powdered if it is
masses. After cooling, add 10 mL of a solution of magne-
sium nitrate hexahydrate in ethanol (1 in 10), and burn the
ethanol. After cooling, add 1 mL of sulfuric acid. Proceed
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 by dissolv-
ing 1.0 g of Latamoxef Sodium in 20 mL of water, and per-
form the test (not more than 2 ppm).
　(4)　Related substances—Dissolve 25 mg of Latamoxef
Sodium in water to make 50 mL, and use this solution as the
sample solution. Pipet 2 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 by the automatic
integration method: the peak area of 1-methyl-1*H*-tetrazole-
5-thiol, having the relative retention time of about 0.5 to the
first eluted peak of the two peaks of latamoxef, obtained
from the sample solution is not larger than the peak area of
latamoxef obtained from the standard solution, and the peak
area of decarboxylatamoxef, having the relative retention
time of about 1.7 to the first peak of the two peaks of
latamoxef, is not larger than 2 times that of latamoxef from
the standard solution. For the peak area of 1-methyl-1*H*-
tetrazole-5-thiol, multiply its ralative response factor, 0.52.
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 5 μL of the standard solution under the above operating
conditions, the relative standard deviation of the peak area
of latamoxef is not more than 2.0%.

Water <*2.48*>　Not more than 5.0% (0.5 g, volumetric titra-
tion, back titration).

Isomer ratio　Dissolve 25 mg of Latamoxef Sodium in water
to make 50 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 fol-
lowing conditions, and determine the areas, A_a and A_b, of
the two peaks in order of elution, which appear close to each
other at the retention time of about 10 minutes: A_a/A_b is be-
tween 0.8 and 1.4.
Operating conditions—
　Detector: An ultraviolet absorption photometer (wave-
length: 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 (10 μm in particle diameter).
　Column temperature: A constant temperature of about
25°C.
　Mobile phase: Dissolve 7.7 g of ammonium acetate in
water to make 1000 mL. To 950 mL of this solution add 50
mL of methanol.
　Flow rate: Adjust so that the retention time of the first
eluted peak of latamoxef is about 8 minutes.
System suitability—
　System performance: When the procedure is run with 5 μL
of the sample solution under the above operating conditions,

the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of latamoxef is not more than 2.0%.

Assay Weigh accurately an amount of Latamoxef Sodium and Latamoxef Ammonium RS, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL of the internal standard solution, add water to make 50 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 latamoxef to that of the internal standard.

Amount [μg (potency)] of latamoxef ($C_{20}H_{20}N_6O_9S$)
= $M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Latamoxef Ammonium RS taken

Internal standard solution—A solution of *m*-cresol (3 in 200).
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 (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 exactly 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust so that the retention time of latamoxef 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, latamoxef 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Not exceeding 5°C.

Lauromacrogol

Polyoxyethylene Lauryl Alcohol Ether

ラウロマクロゴール

Lauromacrogol is a polyoxyethylene ether prepared by the polymerization of ethylene oxide with laury alcohol.

Description Lauromacrogol is a colorless or light yellow, clear liquid or a white, petrolatum-like or waxy solid. It has a characteristic odor, and a somewhat bitter and slightly irritative taste.

It is very soluble in ethanol (95), in diethyl ether and in carbon tetrachloride.

It is freely soluble or dispersed as fine oily drops in water.

Identification **(1)** Shake well 0.5 g of Lauromacrogol with 10 mL of water and 5 mL of ammonium thiocyanate-cobalt nitrate TS, then shake with 5 mL of chloroform, and allow to stand: the chloroform layer becomes blue in color.

(2) Dissolve 0.35 g of Lauromacrogol in 10 mL of carbon tetrachloride, and perform the test as directed in the solution method under Infrared Spectrophotometry <2.25> using a 0.1-mm fixed cell: it exhibits absorption at the wave numbers of about 1347 cm^{-1}, 1246 cm^{-1} and 1110 cm^{-1}.

Purity **(1)** Acidity—Transfer 10.0 g of Lauromacrogol into a flask, and add 50 mL of neutralized ethanol. Heat on a water bath nearly to boil, shaking once or twice while heating. Cool, and add 5.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.
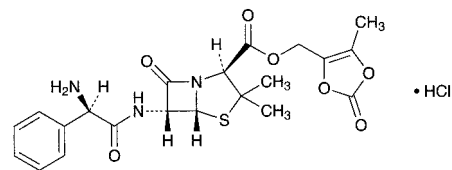
(2) Unsaturated compound—Shake 0.5 g of Lauromacrogol with 10 mL of water, and add 5 drops of bromine TS: the color of the solution does not disappear.

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

Containers and storage Containers—Tight containers.

Lenampicillin Hydrochloride

レナンピシリン塩酸塩

$C_{21}H_{23}N_3O_7S \cdot HCl$: 497.95
5-Methyl-2-oxo[1,3]dioxol-4-ylmethyl (2S,5R,6R)-6-[(2R)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride
[80734-02-7]

Lenampicillin Hydrochloride is the hydrochloride of ampicillin methyloxodioxolenylmethyl ester.

It contains not less than 653 μg (potency) and not more than 709 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvents. The potency of Lenampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Lenampicillin Hydrochloride occurs as a white to light yellowish white powder.

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

Identification **(1)** Determine the infrared absorption spectrum of Lenampicillin 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 Lenampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 mL of a solution of Lenampicillin Hydrochlo-

ride (1 in 100) add 0.5 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $+174 - +194°$ (0.2 g calculated on the anhydrous basis and corrected on the amount of residual solvent, ethanol (95), 20 mL, 100 mm).

Purity (1) Heavy metals $\langle 1.07 \rangle$—Proceed with 2.0 g of Lenampicillin 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 $\langle 1.11 \rangle$—Prepare the test solution with 1.0 g of Lenampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Lenampicillin Hydrochloridein, dissolve in exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. The sample solution should be used to the following test immediately after the solution is prepared. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of ampicillin to that of the internal standard: the amount of ampicillin is not more than 1.0%.

$$\text{Amount (\%) of ampicillin } (C_{16}H_{19}N_3O_4S)$$
$$= M_S/M_T \times Q_T/Q_S \times 2$$

M_S: Amount [mg (potency)] of Ampicillin RS taken
M_T: Amount (mg) of Lenampicillin Hydrochloride taken

Internal standard solution—A solution of anhydrous caffeine in the mobile phase (1 in 50,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 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: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.
Flow rate: Adjust so that the retention time of ampicillin 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, ampicillin 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 ratios of the peak height of ampicillin to that of the internal standard is not more than 5%.
(4) Penicilloic acid—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add 10 mL of potassium hydrogen phthalate buffer solution (pH 4.6) and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 15 minutes while protecting from exposure to light, and titrate

$\langle 2.50 \rangle$ with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction: the amount of penicilloic acid ($C_{16}H_{21}N_3O_5S$: 367.42) is not more than 3.0%.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.45 mg of $C_{16}H_{21}N_3O_5S$

(5) Residual solvent $\langle 2.46 \rangle$—Weigh accurately about 0.25 g of Lenampicillin Hydrochloride, dissolve in exactly 1 mL of the internal standard solution, add N,N-dimethylformamide to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of 2-propanol and about 0.12 g of ethyl acetate, and add N,N-dimethylformamide to make exactly 100 mL. Pipet 1 mL and 3 mL of this solution, add exactly 1 mL each of the internal standard solution, add N,N-dimethylformamide to make 5 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with 4 μL each of the sample solution, standard solution (1) and (2) as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the sample solution, the ratios, Q_{Sa1} and Q_{Sb1}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (1) and the ratios, Q_{Sa2} and Q_{Sb2}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (2). Calculate the amounts of 2-propanol and ethyl acetate by the following equations: not more than 0.7% and not more than 1.7%, respectively.

$$\text{Amount (\%) of 2-propanol}$$
$$= M_{Sa}/M_T \times (2Q_{Ta} - 3Q_{Sa1} + Q_{Sa2})/(Q_{Sa2} - Q_{Sa1})$$

$$\text{Amount (\%) of ethyl acetate}$$
$$= M_{Sb}/M_T \times (2Q_{Tb} - 3Q_{Sb1} + Q_{Sb2})/(Q_{Sb2} - Q_{Sb1})$$

M_{Sa}: Amount (g) of 2-propanol taken
M_{Sb}: Amount (g) of ethyl acetate taken
M_T: Amount (g) of the Lenampicillin Hydrochloride taken

Internal standard solution—A solution of cyclohexane in N,N-dimethylformamide (1 in 1000).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography (180 – 250 μm in particle diameter) coated with tetrakishydroxypropylethylenediamine for gas chromatography at the ratio of 10 to 15%.
Column temperature: A constant temperature of about 80°C.
Injection port temperature: A constant temperature of about 160°C.
Carrier gas: Nitrogen.
Flow rate: Adjust so that the retention time of the internal standard is about 1 minute.
System suitability—
System performance: When the procedure is run with 4 μL of the standard solution (2) under the above operating conditions, the internal standard, ethyl acetate and 2-propanol are eluted in this order, and the resolution between the peaks of the internal standard and ethyl acetate is not less than 2.0.
System repeatability: When the test is repeated 3 times with 4 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak height of ethyl acetate to that of the internal standard is not more than 5.0%.

Water <*2.48*>   Not more than 1.5% (1 g, volumetric titration, direct titration).

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

Assay   Weigh accurately an amount of Lenampicillin Hydrochloride and Lenampicillin Hydrochloride RS, equivalent to about 0.1 g (potency), dissolve each in the internal standard solution to make exactly 10 mL, and use these solutions 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, and calculate the ratios, Q_T and Q_S, of the peak area of lenampicillin to that of the internal standard.

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$)
$$= M_S \times Q_T/Q_S \times 1000$$

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

Internal standard solution—A solution of ethyl aminobenzoate in the mobile phase (1 in 4000).
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 9.53 g of potassium dihydrogen phosphate in water to make exactly 700 mL, and add acetonitrile to make exactly 1000 mL.
   Flow rate: Adjust so that the retention time of lenampicillin 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, lenampicillin 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 lenampicillin to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.
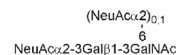
Lenograstim (Genetical Recombination)

レノグラスチム（遺伝子組換え）

Protein moiety

```
TPLGPASSLP QSFLLKCLEQ VRKIQGDGAA LQEKLCATYK LCHPEELVLL

GHSLGIPWAP LSSCPSQALQ LAGCLSQLHS GLFLYQGLLQ ALEGISPELG

PTLDTLQLDV ADFATTIWQQ MEELGMAPAL QPTQGAMPAF ASAFQRRAGG

VLVASHLQSF LEVSYRVLRH LAQP
```

T133: glycosylation site

Carbohydrate moiety (structure of major glycans)

$$(NeuAc\alpha2)_{0,1}$$
$$6$$
$$NeuAc\alpha2\text{-}3Gal\beta1\text{-}3GalNAc$$

$C_{840}H_{1330}N_{222}O_{242}S_8$: 18667.41 (Protein moiety)
[*135968-09-1*]

Lenograstim (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human granulocyte colony-stimulating factor produced in Chinese hamster ovary cells. It is a glycoprotein (molecular mass: ca. 20,000) consisting of 174 amino acid residues. It has a neutrophilic leucocyte induction activity.
   It contains not less than 0.40 mg and not more than 0.60 mg of protein per mL, and not less than 1.02×10^8 units per mg of protein.

Description   Lenograstim (Genetical Recombination) occurs as a clear and colorless liquid.

Identification   **(1)**   Use Lenograstim (Genetical Recombination) and Lenograstim RS as the sample solution and the standard solution, respectively. Perform the test with a volume each of the sample solution and standard solution, equivalent to 20 μg of protein, as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention times of the two peaks of lenograstim in the chromatogram obtained from the sample solution and of those in the chromatogram obtained from the standard solution are the same.
Operating conditions—
   Detector: An ultraviolet absorption photometer (wavelength: 215 nm).
   Column: A stainless steel column 7.5 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group binding synthetic polymer for liquid chromatography (10 μm in particle diameter).
   Column temperature: A constant temperature of about 25°C.
   Mobile phase A: 0.02 mol/L tris buffer solution (pH 7.4).
   Mobile phase B: 0.02 mol/L tris buffer solution (pH 7.4) containing 0.5 mol/L sodium chloride.
   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 – 35	100 → 80	0 → 20
35 – 40	80	20

Flow rate: Adjust so that the retention time of the first appeared peak of lenograstim is about 27 minutes.

System suitability—

System performance: When the procedure is run with a volume of the standard solution, equivalent to 20 μg of protein, under the above operating conditions, the resolution between the two peaks of lenograstim is not less than 4.

(2) Desalt 2 mL each of Lenograstim (Genetical Recombination) and Lenograstim RS by a suitable method, and assign them as the desalted sample and the desalted reference standard, respectively. Add the desalted sample and the desalted reference standard in 100 μL each of a mixture of water and 1-propanol (3:2), add 4 mL of urea-EDTA TS, and allow them to stand at 37°C for 18 hours. Then, add 10 μL of 2-mercaptoethanol to them, and allow to stand at 37°C for 4 hours. To these solutions add a solution of 27 mg of iodoacetic acid in 150 μL of sodium hydroxide TS, and react at 37°C for 15 minutes, avoiding exposure to light. Remove the reagents from these reaction solution by a suitable method, and assign obtained these substances as the reduced carboxymethylated sample and the reduced carboxymethylated reference standard. To these substances add 100 μL each of a mixture of water and 1-propanol (3:2), and add 1 mL of 0.05 mol/L ammonium hydrogen carbonate solution. Add 20 μL each of a solution of V8 protease in 0.05 mol/L ammonium hydrogen carbonate solution (1 in 1000), and react at 37°C for 18 hours. To each reaction solution add 50 μL of diluted trifluoroacetic acid (1 in 10) to stop the reaction, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 100 μL – 150 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 from these solutions: both chromatograms show the similar peaks at the corresponding retention time.

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 25°C.

Mobile phase A: A mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (950:50:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water, and trifluoroacetic acid (800:200:1).

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 – 120	100 → 20	0 → 80
120 – 140	20 → 0	80 → 100
140 – 150	0	100

Flow rate: Adjust so that the retention time of the first appeared peak is about 33 minutes.

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the first appeared peak and the second appeared peak is not less than 15.

Monosaccharide composition Put exactly 2 mL of Lenograstim (Genetical Recombination) into a precolumn, packed with 0.36 g of octadecylsilanized silica gel for pretreatment, wash the column with 5 mL of a mixture of water, acetonitrile and trifluoroacetic acid (600:400:1), then elute with a mixture of acetonitrile, water and trifluoroacetic acid (800:200:1), and collect exactly 5 mL of the first eluate. Pipet 1.5 mL of the eluate in a test tube, add exactly 20 μL of the internal standard solution, and lyophilize. Dissolve the lyophilized substance in 250 μL of a mixture of methanol and acetyl chloride (9:1), seal the tube, and heat at 90°C for 2 hours. After cooling, open the tube, and dry the content under reduced pressure. To the residue add 200 μL of methanol, and evaporate to dryness under reduced pressure. Dissolve the residue in 200 μL of a solution of pyridine in methanol (1 in 10) and 50 μL of acetic anhydride, stopper the tube tightly, and allow to stand for 10 minutes. Evaporate the solution to dryness at about 50°C under reduced pressure, add 200 μL of methanol to the residue, and evaporate to dryness at 50°C under reduced pressure. To the residue add 50 μL of a mixture of pyridine, 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsilane (10:2:1), stopper tightly, shake vigorously for 30 seconds, and warm at 50°C for 10 minutes. After cooling, add 300 μL of pentane, stir gently, then add 300 μL of water, and stir gently. Separate the upper layer, evaporate to concentrate to about 10 μL under a stream of nitrogen, and use this as the sample solution. Separately, weigh accurately about 54 mg of D-galactose and about 33 mg of N-acetylgalactosamine, dissolve them separately in water to make exactly 20 mL each, and use these solutions as D-galactose solution and N-acetylgalactosamine solution, respectively. Weigh accurately about 9.3 mg of N-acetylneuraminic acid, add exactly 1 mL of the D-galactose solution and exactly 2 mL of the N-acetylgalactosamine solution to dissolve, and add water to make exactly 20 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution, and freeze-dry 40 μL of this solution. Dissolve the freeze-dried substance in 250 μL of a mixture of methanol and acetyl chloride (9:1), then proceed in the same manner as the sample solution, and use the solution obtained as the monosaccharide standard solution. Perform the test with 2 μL each of the sample solution and the monosaccharide standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios of each peak area of D-galactose, N-acetylgalactosamine and N-acetylneuraminic acid to that of the internal standard, Q_T and Q_S. Calculate the amount (mol/mol of lenograstim) of each monosaccharide by the following formula: the amounts of D-galactose, N-acetylgalactosamine and N-acetylneuraminic acid are between 0.7 and 1.2, between 0.7 and 1.2, and between 1.0 and 2.0, respectively.

Amount (mol/mol of lenograstim) of each monosaccharide
$$= M/(M_m \times D_S) \times Q_T/Q_S \times 18{,}667/C \times 5/3$$

M: Amount (mg) of each monosaccharide taken
M_m: Molecular mass of each monosaccharide
    D-galactose: 180.16
    N-acetylgalactosamine: 221.21
    N-acetylneuraminic acid: 309.27
D_S: Dilution rate of each monosaccharide
    D-galactose: 20,000

N-acetylgalactosamine and: 10,000

N-acetylneuraminic acid: 1000

C: Protein concentration (mg/mL) of Lenograstim (Genetical Recombination)

18,667: Molecular mass of protein moiety of lenograstim

Internal standard solution—Dissolve 48 mg of myoinositol in water to make 50 mL. To 1 mL of this solution add water to make 20 mL.

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 7% cyanopropyl-7% phenyl-methyl silicon polymer for gas chromatography 0.25 μm in thickness.

Column temperature: Rise the temperature at a rate of 10°C per minute from 110°C to 185°C, then at a rate of 2°C per minute to 210°C, and to 260°C at a rate of 8°C per minute, and maintain 260°C for 15 minutes.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of the internal standard is about 24 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the monosaccharide standard solution under the above operating conditions, D-galactose, the internal standard, *N*-acetylgalactosamine and *N*-acetylneuraminic acid are eluted in this order, and the resolution between the peaks of the internal standard and *N*-acetylgalactosamine is not less than 10.

pH *<2.54>*   7.7 – 8.3

Purity (1) Related substances—Perform the test with a volume of Lenograstim (Genetical Recombination), equivalent to 30 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 the amount of these peaks by the area percentage method excluding the area of the solvent peak: the total amount of the peaks other than lenograstim is not more than 1.0%.

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 porous silica gel for liquid chromatography (10 μm in particle diameter).

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

Mobile phase: Dissolve 1.4 g of anhydrous disodium hydrogen phosphate and 5.8 g of sodium chloride in water to make 1000 mL (Solution A). Separately, dissolve 1.6 g of sodium dihydrogen phosphate dihydrate and 5.8 g of sodium chloride in water to make 1000 mL (Solution B). Adjust the pH of Solution A to 7.4 with Solution B.

Flow rate: Adjust so that the retention time of lenograstim is about 21 minutes.

Time span of measurement: About 2 times as long as the retention time of lenograstim.

System suitability—

Test for required detectability: When the procedure is run with 60 μL of diluted Lenograstim RS with the solvent of Lenograstim (Genetical Recombination) containing 0.1 vol% polysorbate 20 (1 in 500) under the above operating conditions, the peak of lenograstim is detectable.

System performance: When the procedure is run using Lenograstim RS under the above operating conditions, the number of theoretical plates of the peak of lenograstim is not less than 2700.

(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—Use Lenograstim (Genetical Recombination) and Lenograstim RS as the sample solution and the standard solution, respectively. Perform the test with exactly 30 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 lenograstim in each solution.

Amount (mg) of protein in 1 mL of Lenograstim (Genetical Recombination)
$$= C_S \times A_T/A_S$$

C_S: Concentration (mg/mL) of protein in Lenograstim RS

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 25°C.

Mobile phase A: A mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (600:400:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water and trifluoroacetic acid (800:200:1).

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 – 40	80 → 30	20 → 70

Flow rate: Adjust so that the retention time of lenograstim is about 35 minutes.

System suitability—

System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of lenograstim is not less than 2900.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lenograstim is not more than 4.0%.

(2) Specific activity—Dilute Lenograstim (Genetical Recombination) with FBS-IMDM so that each mL contains an estimate amount of 7.69 units, 10.0 units and 13.0 units, and name them as the sample solution (1), the sample solution (2) and the sample solution (3), respectively. Separately, dilute Lenograstim RS with FBS-IMDM so that each mL contains 7.69 units, 10.0 units and 13.0 units, and name them as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Put exactly 100 μL each of the sample solutions and standard solutions in wells of a sterile disposable multiple well plate, add 50 μL each of NFS-60 cell suspension (prepared by adding FBS-IMDM so the each mL contains about 5 × 10⁵ cells) to each well and mix to make homogenize, and place the plate in a CO_2 incubator at 37°C. After incubation for 22 hours, add 15 μL of resazurin solution to each well, and determine the

absorbances at 570 nm, A_{T1} and A_{S1}, and at 600 nm, A_{T2} and A_{S2}. From the reaction values at each concentration of the standard solution and sample solution [deference of absorbance ($A_{S1} - A_{S2}$ and $A_{T1} - A_{T2}$)], determine the rate of potency (Pr) of the sample solution to the standard solution by the parallel assay, and calculate the potency (unit) per 1 mg of protein of Lenograstim (Genetical Recombination).

Pr = anti ln (M)
$M = (P_T - P_S)/db$
$P_T = T_1 + T_2 + T_3$
$P_S = S_1 + S_2 + S_3$
b = $H_L(L_S + L_T)/I$nh
$H_L = 12n/(d^3 - d)$
$L_S = 1S_1 + 2S_2 + 3S_3 - 1/2(d + 1)P_S$
$L_T = 1T_1 + 2T_2 + 3T_3 - 1/2(d + 1)P_T$
d = 3
I = ln 1.3
n = 3
h = 2

T_1: Mean of reaction values of the sample solution (1)
T_2: Mean of reaction values of the sample solution (2)
T_3: Mean of reaction values of the sample solution (3)
S_1: Mean of reaction values of the standard solution (1)
S_2: Mean of reaction values of the standard solution (2)
S_3: Mean of reaction values of the standard solution (3)

Specific activity (unit/mg of protein) of lenograstim
$= S \times Pr \times D_T/D_S/C$

S: Potency (unit/mL) of Lenograstim RS
D_T: Dilution rate of the sample solution (3)
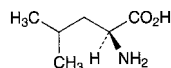D_S: Dilution rate of the standard solution (3)
C: Concentration (mg/mL) of protein of sample

Containers and storage Containers—Tight containers.
Storage—At a temperature not exceeding $-20°C$.

L-Leucine

L-ロイシン

$C_6H_{13}NO_2$: 131.17
(2*S*)-2-Amino-4-methylpentanoic acid
[*61-90-5*]

L-Leucine, when dried, contains not less than 98.5% of L-leucine ($C_6H_{13}NO_2$).

Description L-Leucine 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-Leucine, 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> $[\alpha]_D^{20}$: $+14.5 - +16.0°$ (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Leucine 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-Leucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Leucine in 40 mL of water and 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 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Leucine in 40 mL of water and 1 mL of dilute hydrochloric acid, and add water to make 50 mL. 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 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Leucine. 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 L-Leucine 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).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Leucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Leucine in water by warming, after cooling, add water to make 25 mL, 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 a solution of ninhydrin in acetone (1 in 50) on the plate, 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-Leucine, 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—Well-closed containers.

Leuprorelin Acetate

リュープロレリン酢酸塩
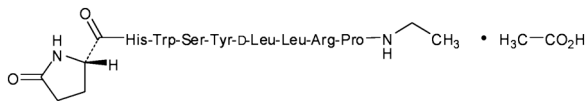
C$_{59}$H$_{84}$N$_{16}$O$_{12}$.C$_2$H$_4$O$_2$: 1269.45
5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide monoacetate
[*74381-53-6*]

Leuprorelin Acetate contains not less than 96.0% and not more than 102.0% of leuprorelin (C$_{59}$H$_{84}$N$_{16}$O$_{12}$: 1209.40), calculated on the anhydrous and residual acetic acid-free basis.

Description Leuprorelin Acetate occurs as a white to yellowish white powder.

It is very soluble in water and in acetic acid (100), freely soluble in methanol, and sparingly soluble in ethanol (99.5).

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Leuprorelin 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 Leuprorelin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]$_D^{20}$: −38 – −41° (0.25 g calculated on the anhydrous and residual acetic acid-free basis, diluted acetic acid (100) (1 in 100), 25 mL, 100 mm).

pH <2.54> The pH of a solution of 0.10 g of Leuprorelin Acetate in 10 mL of water is 5.5 to 7.5.

Constituent amino acids When hydrolyzed by Method 1 described in "1. Hydrolysis of Protein and Peptide" and performed the test by Method 1 described in "2. Methodologies of Amino Acid Analysis" under Amino Acid Analysis of Proteins <2.04>, histidine, glutamic acid, proline, tyrosine and arginine is 1 and leucine is 2, respectively.
Procedure

(i) Hydrolysis Weigh accurately about 50 mg of Leuprorelin Acetate, and dissolve in 1 mL of water. Put 0.1 mL of this solution in a test tube for hydrolysis, freeze-dry the content, and add 2 mL of a solution of phenol in 6 mol/L hydrochloric acid (1 in 200). Freeze the solution, seal the tube in vacuum, and heat the tube at 110°C for 24 hours. After cooling, open the tube, take out 0.1 mL of the hydrolyzate, add 1 mL of water, and freeze-dry. Dissolve the residue in 7.8 mL of diluting solution, and use this solution as the sample solution. Separately, weigh exactly 0.45 mg of L-alanine, 0.66 mg of L-aspartic acid, 1.05 mg of L-arginine hydrochloride, 0.74 mg of L-glutamic acid, 0.38 mg of glycine, 1.05 mg of L-histidine hydrochloride monohydrate, 0.66 mg of L-isoleucine, 0.66 mg of L-leucine, 0.58 mg of L-proline, 0.53 mg of L-serine, 0.60 mg of L-threonine and 0.91 mg of L-tyrosine, dissolve in diluting solution to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 1 mg of L-tryptophan and 0.4 mg of ethylamine hydrochloride in diluting solution to make 100 mL, and use this solution as the standard solution (2).

(ii) Amino acid analysis Perform the test with exactly 100 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions: the peaks of histidine, glutamic acid, leucine, proline, tyrosine, arginine, serine and tryptophan appear on the chromatogram obtained from the sample solution. Apart from this, calculate the molar content of each constituent amino acid in 1 mL of the sample solution from the peak area of each amino acid obtained from the sample solution and standard solution (1), and further calculate the number of the constituent amino acids assuming that the sum of each molar content of histidine, glutamic acid, leucine, proline, tyrosine and arginine in 1 mole of leuprorelin acetate is 7.

Diluting solution: Dissolve 6.29 g of lithium hydroxide monohydrate and 10.51 g of citric acid monohydrate in water to make 1000 mL, and adjust to pH 2.2 with hydrochloric acid.
Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 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 (Na type) for liquid chromatography (3 μm in particle diameter).

Column temperature: Maintain a constant temperature of about 58°C for 18 minutes after injection, then maintain a constant temperature of about 70°C for a further 20 minutes.

Reaction vessel temperature: A constant temperature of about 135°C.

Mobile phase: Prepare the mobile phases A, B, C, D and E according to the following table, then add 0.1 mL of 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	20.0 mL	4.0 mL	—	100 mL
Thiodiglycol	5.0 mL	5.0 mL	5.0 mL	—	—
Benzyl alcohol	—	—	—	5.0 mL	—
Lauromacrogol solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 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

Flowing of mobile phase: Control the gradient by mixing the mobile phases A, B, C, D and E 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%)	Mobile phase E (vol%)
0 – 1.6	100	0	0	0	0
1.6 – 4.5	0	100	0	0	0
4.5 – 13.5	0	0	100	0	0
13.5 – 27.0	0	0	0	100	0
27.0 – 33.0	0	0	0	0	100

Reaction reagent: Dissolve an appropriate amount of lithium acetate dihydrate, acetic acid (100) and 1-methoxy-2-propanol in water to make 1000 mL, and use this solution as solution A. Separately, dissolve an appropriate amount of ninhydrin and sodium borohydride in 1-methoxy-2-propanol to make 1000 mL, and use this solution as solution B. Mix equal parts of solutions A and B before use.

Flow rate of mobile phase: About 0.40 mL per minute.

Flow rate of reaction reagent: About 0.35 mL per minute.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution (1) 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 1.2, respectively.

System repeatability: When the test is repeated 5 times with 100 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of arginine, aspartic acid, proline and serine is not more than 4.0%.

Purity   Related substances—Dissolve 0.10 g of Leuprorelin Acetate in the mobile phase to make 100 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 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 peaks, having the relative retention time of about 0.65, about 0.77, about 0.78 and about 0.90 to leuprorelin, obtained from the sample solution is not larger than 1/2 times the peak area of leuprorelin obtained from the standard solution, and the total area of the peaks other than leuprorelin from the sample solution is not larger than 2 times the peak area of leuprorelin 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 leuprorelin, 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 exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of leuprorelin 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.

Water <2.48>   Not more than 5.0% (0.1 g, coulometric titration).

Residue on ignition <2.44>   Not more than 0.2% (0.5 g).

Acetic acid   Weigh accurately about 0.1 g of Leuprorelin Acetate, dissolve in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of acetic acid (100), 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 the peak areas, A_T and A_S, of acetic acid in each solution, and calculate the amount of acetic acid by the following equation: 4.7 – 8.0%.

$$\text{Amount (\%) of acetic acid} = M_S/M_T \times A_T/A_S \times 10$$

M_S: Amount (g) of acetic acid (100) taken
M_T: Amount (g) of Leuprorelin Acetate taken

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 25°C.

Mobile phase: To 0.7 mL of phosphoric acid add water to make 1000 mL, and adjust to pH 3.0 with a solution of sodium hydroxide (21 in 50). To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust so that the retention time of acetic acid is 3 to 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the symmetry factor of the peak of acetic acid is not more 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 acetic acid is not more than 2.0%.

Assay   Weigh accurately about 0.1 g each of Leuprorelin Acetate and Leuprorelin Acetate RS (separately determine the water <2.48> and acetic acid in the same manner as Leuprorelin Acetate), dissolve separately in the mobile phase to make exactly 100 mL. To exactly 5 mL each of these solutions add the mobile phase to make them exactly 100 mL, and use so obtained 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 leuprorelin in each solution.

$$\text{Amount (mg) of leuprorelin } (C_{59}H_{84}N_{16}O_{12})$$
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of Leuprorelin Acetate RS taken, calculated on the anhydrous and de-acetic acid basis

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: Dissolve 15.2 g of triethylamine in 800 mL of water, adjust to pH 3.0 with phosphoric acid, and add

water to make 1000 mL. To 850 mL of this solution add 150 mL of a mixture of acetonitrile and 1-propanol (3:2).

Flow rate: Adjust so that the retention time of leuprorelin is 41 to 49 minutes (1.0 – 1.5 mL per minute).

System suitability—

System performance: Dissolve about 0.1 g of Leuprorelin Acetate RS in 100 mL of the mobile phase. To 5 mL of this solution add water to make 50 mL. To 5 mL of this solution add 0.1 mL of sodium hydroxide TS, stopper the vessel, shake vigorously, then heat at 100°C for 60 minutes. After cooling, add 50 μL of 1 mol/L phosphoric acid solution, and shake vigorously. When the procedure is run with 20 μL of this solution under the above operating conditions, a peak having the relative retention time of about 0.90 to leuprorelin and leuprorelin are eluted in this order with the resolution between these peaks being not less than 1.5.

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 peak area of leuprorelin is not more than 1.5%.

Containers and storage   Containers—Hermetic containers.

Levallorphan Tartrate

レバロルファン酒石酸塩
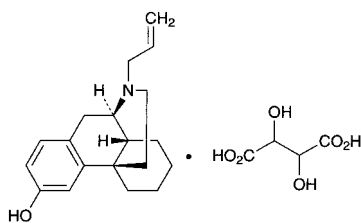
C$_{19}$H$_{25}$NO.C$_4$H$_6$O$_6$: 433.49
17-Allylmorphinan-3-ol monotartrate
[*71-82-9*]

Levallorphan Tartrate, when dried, contains not less than 98.5% of levallorphan tartrate (C$_{19}$H$_{25}$NO. C$_4$H$_6$O$_6$).

Description   Levallorphan Tartrate occurs as a white to pale yellow crystalline powder. It is odorless.

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

Identification   (1)   Determine the absorption spectrum of a solution of Levallorphan Tartrate in 0.01 mol/L hydrochloric acid TS (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 Levallorphan Tartrate, 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 Levallorphan Tartrate (1 in 30) responds to the Qualitative Tests <*1.09*> (1) and (2) for tartrate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −37.0 – −39.2° (after drying, 0.2 g, water, 10 mL, 100 mm).

pH <*2.54*>   Dissolve 0.2 g of Levallorphan Tartrate in 20 mL of water: the pH of this solution is between 3.3 and 3.8.

Melting point <*2.60*>   174 – 178°C

Purity   (1)   Clarity and color of solution—Dissolve 0.2 g of Levallorphan Tartrate in 10 mL of water: the solution is clear and colorless.

(2)   Heavy metals <*1.07*>—Proceed with 1.0 g of Levallorphan Tartrate 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 Levallorphan Tartrate 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 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 for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia TS (200:3) 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% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

Residue on ignition <*2.44*>   Not more than 0.10% (1 g).

Assay   Weigh accurately about 0.5 g of Levallorphan Tartrate, previously dried, dissolve in 30 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
= 43.35 mg of C$_{19}$H$_{25}$NO.C$_4$H$_6$O$_6$

Containers and storage   Containers—Well-closed containers.

Levallorphan Tartrate Injection

レバロルファン酒石酸塩注射液

Levallorphan Tartrate Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of levallorphan tartrate (C$_{19}$H$_{25}$NO.C$_4$H$_6$O$_6$: 433.49).

Method of preparation   Prepare as directed under Injection, with Levallorphan Tartrate.

Description   Levallorphan Tartrate Injection is a clear, colorless liquid.
pH: 3.0 – 4.5

Identification   Take an exact volume of Levallorphan Tartrate Injection, equivalent to 3 mg of Levallorphan Tartrate, add 5 mL of water and 2 drops of dilute hydrochloric acid, and wash with five 15-mL portions of diethyl ether by a vigorous shaking. Take the water layer, evaporate the diethyl ether remained by warming on a water bath, and after cooling, add 0.01 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 277 nm and 281 nm.

Bacterial endotoxins <*4.01*>   Less than 150 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 Take exactly a volume of Levallorphan Tartrate Injection, equivalent to about 2 mg of levallorphan tartrate ($C_{19}H_{25}NO.C_4H_6O_6$), add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of levallorphan tartrate for assay, previously dried at 80°C for 4 hours on phosphorus (V) oxide under reduced pressure, and dissolve in water to make exactly 100 mL. Pipet 2 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 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 levallorphan to that of the internal standard:

Amount (mg) of levallorphan tartrate ($C_{19}H_{25}NO.C_4H_6O_6$)
$$= M_S \times Q_T/Q_S \times 1/50$$

M_S: Amount (mg) of levallorphan tartrate for assay taken

Internal standard solution—Dissolve 0.04 g of isobutyl parahydroxybenzoate in 10 mL of ethanol (95), add water to make 100 mL, and to 10 mL of this solution add water to make 100 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 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 300 mL of this solution add 200 mL of acetonitrile.
Flow rate: Adjust so that the retention time of levallorphan 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, the internal standard and levallorphan 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 ratios of the peak area of levallorphan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Levodopa

レボドパ
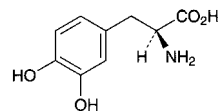
$C_9H_{11}NO_4$: 197.19
3-Hydroxy-L-tyrosine
[*59-92-7*]

Levodopa, when dried, contains not less than 98.5% of levodopa ($C_9H_{11}NO_4$).

Description Levodopa occurs as white or slightly grayish white, crystals or crystalline powder. It is odorless.
It is freely soluble in formic acid, slightly soluble in water, and practically insoluble in ethanol (95).
It dissolves in dilute hydrochloric acid.
The pH of a saturated solution of Levodopa is between 5.0 and 6.5.
Melting point: about 275°C (with decomposition).

Identification **(1)** To 5 mL of a solution of Levodopa (1 in 1000) add 1 mL of ninhydrin TS, and heat for 3 minutes in a water bath: a purple color develops.
(2) To 2 mL of a solution of Levodopa (1 in 5000) add 10 mL of 4-aminoantipyrine TS, and shake: a red color develops.
(3) Dissolve 3 mg of Levodopa in 0.001 mol/L hydrochloric acid 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: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance *<2.24>* $E_{1\,cm}^{1\%}$ (280 nm): 136 – 146 (after drying, 30 mg, 0.001 mol/L hydrochloric acid TS, 1000 mL).

Optical rotation *<2.49>* $[\alpha]_D^{20}$: -11.5 – $-13.0°$ (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Levodopa in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.
(2) Chloride *<1.03>*—Dissolve 0.5 g of Levodopa 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 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).
(3) Sulfate *<1.14>*—Dissolve 0.40 g of Levodopa in 1 mL of dilute hydrochloric acid and 30 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 0.25 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).
(4) Heavy metals *<1.07>*—Proceed with 1.0 g of Levodopa 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>*—Dissolve 1.0 g of Levodopa in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).
(6) Related substances—Dissolve 0.10 g of Levodopa in 10 mL of sodium disulfite TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium disulfite TS to make exactly 25 mL. Pipet 1 mL of this solution, add sodium disulfite TS 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 cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and methanol (10:5:5:1) 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 0.30% (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 Levodopa, previously dried, dissolve in 3 mL of formic acid, add 80 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-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 19.72 mg of $C_9H_{11}NO_4$

</div>

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

Levofloxacin Hydrate

レボフロキサシン水和物
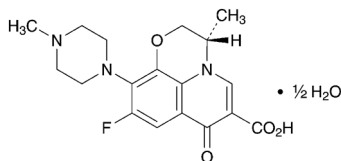
$C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$: 370.38
(3*S*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid hemihydrate
[*138199-71-0*]

Levofloxacin Hydrate contains not less than 99.0% and not more than 101.0% of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37), calculated on the anhydrous basis.

Description   Levofloxacin Hydrate occurs as light yellowish white to yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually turns dark light yellowish white on exposure to light.

Melting point: about 226°C (with decomposition).

Identification   (1)   Determine the absorption spectrum of a solution of Levofloxacin Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 150,000) 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 Levofloxacin 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.

Optical rotation ⟨2.49⟩ $[\alpha]_D^{20}$: −92 – −99° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity   (1)   Heavy metals ⟨1.07⟩—Proceed with 2.0 g of Levofloxacin 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—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Levofloxacin Hydrate in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (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 both solutions by the automatic integration method: the area of the peak having the relative retention time of about 1.2 to levofloxacin obtained from the sample solution is not larger than 2/5 times the peak area of levofloxacin obtained from the standard solution, and the area of each peak other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 to levofloxacin from the sample solution is not larger than 1/5 times the peak area of levofloxacin from the standard solution. Furthermore, the total area of the peaks other than levofloxacin and the peak having the relative retention time of about 1.2 to levofloxacin from the sample solution is not larger than 3/10 times the peak area of levofloxacin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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: Dissolve 1.76 g of L-valine, 7.71 g of ammonium acetate and 1.25 g of Copper (II) sulfate pentahydrate in water to make 1000 mL. To this solution add 250 mL of methanol.

Flow rate: Adjust so that the retention time of levofloxacin is about 22 minutes.

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of levofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6% of that of levofloxacin obtained from 10 μL of the standard solution.

System performance: Dissolve 10 mg of ofloxacin in 20 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add a mixture of water and methanol (1:1) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of levofloxacin and the peak having the relative retention time of about 1.2 to levofloxacin is 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 levofloxacin is not more than 3.0%.

Water *<2.48>* 2.1 – 2.7% (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 Levofloxacin Hydrate, 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 in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $C_{18}H_{20}FN_3O_4$

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

Levofloxacin Fine Granules

レボフロキサシン細粒

Levofloxacin Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37).

Method of preparation Prepare as directed under Granules, with Levofloxacin Hydrate.

Identification To an amount of Levofloxacin Fine Granules, equivalent to 50 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 50 mL, and stir for 20 minutes. 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 to 1 mL of the subsequent filtrate add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits maxima between 225 nm and 229 nm and between 292 nm and 296 nm, and a shoulder between 321 nm and 331 nm.

Uniformity of dosage units *<6.02>* Perform the test according to the following method: the Granules in single-dose packages meet the requirement of the Content uniformity test.

To the total amount of the content of 1 package of Levofloxacin Fine Granules add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly V mL so that each mL contains about 1 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), and stir for 20 minutes. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of levofloxacin hydrate for assay (separately determine the water *<2.48>* in the same manner as Levofloxacin Hydrate), and dissolve in diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 327 nm of the sample solution and standard solution as directed under Ultraviolet-

visible Spectrophotometry *<2.24>*.

Amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$)
= $M_S \times A_T/A_S \times V/25$

M_S: Amount (mg) of levofloxacin hydrate 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 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Levofloxacin Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Levofloxacin Fine Granules, equivalent to about 0.1 g of levofloxacin ($C_{18}H_{20}FN_3O_4$), 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 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of levofloxacin hydrate for assay (separately determine the water *<2.48>* in the same manner as Levofloxacin Hydrate), 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. Determine the absorbances, A_T and A_S, at 289 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 levofloxacin ($C_{18}H_{20}FN_3O_4$)
= $M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount (mg) of levofloxacin hydrate for assay taken, calculated on the anhydrous basis
M_T: Amount (g) of Levofloxacin Fine Granules taken
C: Labeled amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$) in 1 g

Assay Weigh accurately an amount of Levofloxacin Fine Granules, powder if necessary, equivalent to about 50 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL, stir for 20 minutes, and filter this solution 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 diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of levofloxacin hydrate for assay (separately determine the water *<2.48>* in the same manner as Levofloxacin Hydrate), and dissolve in diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL. Pipet 5 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) 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 levofloxacin in each solution.

Amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$)
= $M_S \times A_T/A_S$

M_S: Amount (mg) of levofloxacin hydrate for assay taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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: Dissolve 1.00 g of copper (II) sulfate pentahydrate, 1.41 g of L-valine and 6.17g of ammonium acetate in 800 mL of water, and add 200 mL of methanol.

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

System suitability—

System performance: Dissolve 10 mg of ofloxacin in 20 mL of diluted 3 mol/L hydrochloric acid TS (1 in 100). To 1 mL of this solution add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, levofloxacin and an enantiomer 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 levofloxacin is not more than 1.0%.

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

Levofloxacin Injection

レボフロキサシン注射液

Levofloxacin Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37).

Method of preparation   Prepare as directed under Injections, with Levofloxacin Hydrate.

Description   Levofloxacin Injection is yellow to greenish yellow, clear liquid.

Identification   To a volume of Levofloxacin Injection, equivalent to 50 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 1 mol/L hydrochloric acid TS (3 in 100) to make 50 mL. To 1 mL of this solution add diluted 1 mol/L hydrochloric acid TS (3 in 100) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 225 nm and 229 nm and between 292 nm and 296 nm, and a shoulder between 321 nm and 331 nm.

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

Bacterial endotoxin <4.01>   Less than 0.60 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   To an exact volume of Levofloxacin Injection, equivalent to about 50 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 1 mol/L hydrochloric acid TS (3 in 100) to make exactly 50 mL. Pipet 5 mL of this solution, add diluted 1

mol/L hydrochloric acid TS (3 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of levofloxacin hydrate for assay (separately determine the water <2.48> in the same manner as Levofloxacin Hydrate), and dissolve in diluted 1 mol/L hydrochloric acid TS (3 in 100) to make exactly 50 mL. Pipet 5 mL of this solution, add diluted 1 mol/L hydrochloric acid TS (3 in 100) 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 levofloxacin in each solution.

$$\text{Amount (mg) of levofloxacin } (C_{18}H_{20}FN_3O_4)$$
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of levofloxacin for assay taken, calculated on the anhydrous basis

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (2) under Levofloxacin Hydrate.

Mobile phase: Dissolve 1.41 g of L-valine, 6.17 g of ammonium acetate, and 1.00 g of copper (II) sulfate pentahydrate in 800 mL of water, and add 200 mL of methanol.

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

System suitability—

System performance: Dissolve 10 mg of ofloxacin in 20 mL of diluted 1 mol/L hydrochloric acid TS (3 in 100). To 1 mL of this solution add diluted 1 mol/L hydrochloric acid TS (3 in 100) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, levofloxacin and the enantiomer 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 levofloxacin is not more than 1.0%.

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

Levofloxacin Ophthalmic Solution

レボフロキサシン点眼液

Levofloxacin 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 levofloxacin hydrate ($C_{18}H_{20}FN_3O_4.\frac{1}{2}H_2O$: 370.38).

Method of preparation   Prepare as directed under Ophthalmic Liquids and Solutions, with Levofloxacin Hydrate.

Description   Levofloxacin Ophthalmic Solution occurs as a clear, pale yellow to yellow liquid.

Identification   **(1)**   To a volume of Levofloxacin Ophthalmic Solution, equivalent to 5 mg of Levofloxacin Hydrate, add 0.01 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.01 mol/L hydrochloric acid TS to make 20 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 maxima between 225 nm and 229 nm, and between 292 nm and 296 nm.

(2) To a volume of Levofloxacin Ophthalmic Solution, equivalent to 5 mg of Levofloxacin Hydrate, add a mixture of water and methanol (1:1) to make 5 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of levofloxacin hydrate 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the principal peaks in the chromatogram obtained from the sample solution and the standard solution is the same.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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: Dissolve 1.25 g of copper (II) sulfate pentahydrate, 1.76 g of L-valine and 7.71 g of ammonium acetate in water to make 1000 mL, and add 250 mL of methanol.

Flow rate: Adjust so that the retention time of levofloxacin is about 22 minutes.

System suitability—

System performance: Dissolve 10 mg of ofloxacin in 20 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add a mixture of water and methanol (1:1) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of levofloxacin and the peak having the relative retention time of about 1.2 to levofloxacin is not less than 3.

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.

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 To an exact volume of Levofloxacin Ophthalmic Solution, equivalent to about 5 mg of levofloxacin hydrate ($C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$) add exactly 2 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 levofloxacin hydrate for assay (separately determine the water <2.48> in the same manner as Levofloxacin Hydrate), and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, then add 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 levofloxacin to that of the internal standard.

Amount (mg) of levofloxacin hydrate ($C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$)
  = $M_S \times Q_T/Q_S \times 1/5 \times 1.025$

M_S: Amount (mg) of levofloxacin hydrate for assay taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of naphazoline hydrochloride in the mobile phase (3 in 500).

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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 13.61 g of potassium dihydrogen phosphate and 0.77 g of ammonium acetate in 900 mL of water, adjust to pH 3.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL. To 900 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust so that the retention time of levofloxacin is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, levofloxacin 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 levofloxacin to that of the internal standard is not more than 1.0%.

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

Levofloxacin Tablets

レボフロキサシン錠

Levofloxacin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37).

Method of preparation Prepare as directed under Tablets, with Levofloxacin Hydrate.

Identification To an amount of powdered Levofloxacin Tablets, equivalent to 0.1 g of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 100 mL, and stir for 20 minutes. 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 to 1 mL of the subsequent filtrate add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 225 nm and 229 nm and between 292 nm and 296 nm, and a shoulder between 321 nm and 331 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 Levofloxacin Tablets add about 70 mL of diluted 3 mol/L hydrochloric acid TS (1 in 100), agitate to disintegrate the tablet with the aid of ultrasonic waves, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and stir for 20 minutes. Pipet V mL the solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to

make exactly V' mL so that each mL contains about 50 μg of levofloxacin ($C_{18}H_{20}FN_3O_4$), and 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. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of levofloxacin } (C_{18}H_{20}FN_3O_4)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/5$$

M_S: Amount (mg) of levofloxacin hydrate for assay taken, calculated on the anhydrous basis

Dissolution $\langle 6.10 \rangle$ **(1)**   For a 100-mg Tablet   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 is not less than 80%.

Start the test with 1 tablet of Levofloxacin 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 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of levofloxacin hydrate for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Levofloxacin Hydrate), 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. Determine the absorbances, A_T and A_S, at 289 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$.

Dissolution rate (%) with respect to the labeled amount of levofloxacin hydrate ($C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$)
$$= M_S \times A_T/A_S \times 18/5 \times 1.025$$

M_S: Amount (mg) of levofloxacin hydrate for assay taken, calculated on the anhydrous basis

(2)   For a 250-mg Tablet and 500-mg Tablet   When the tests are 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 30 minutes is not less than 80%.

Start the test with 1 tablet of Levofloxacin 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 11.2 μg of levofloxacin ($C_{18}H_{20}FN_3O_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of levofloxacin hydrate for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Levofloxacin Hydrate), and dissolve in the dissolution medium to make exactly 50 mL. Pipet 2 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, at 287 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$.

Dissolution rate (%) with respect to the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$$

M_S: Amount (mg) of levofloxacin hydrate for assay taken, calculated on the anhydrous basis
C: Amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$) in 1 g

Assay   Accurately weigh the mass of not less than 20 Levofloxacin Tablets, and powder them. Weigh accurately a portion of the powder, equivalent to about 1 g of levofloxacin ($C_{18}H_{20}FN_3O_4$), add 150 mL of diluted 3 mol/L hydrochloric acid TS (1 in 100), agitate with the aid of ultrasonic waves for 5 minutes, and add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 200 mL, and stir for 10 minutes. Pipet 2 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 200 mL, 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 25 mg of levofloxacin hydrate for assay (separately determine the water $\langle 2.48 \rangle$ in the same manner as Levofloxacin Hydrate), and dissolve in diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) 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 $\langle 2.01 \rangle$ according to the following conditions, and determine the peak areas, A_T and A_S, of levofloxacin in each solution.

$$\text{Amount (mg) of levofloxacin } (C_{18}H_{20}FN_3O_4)$$
$$= M_S \times A_T/A_S \times 40$$

M_S: Amount (mg) of levofloxacin hydrate for assay taken, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 340 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: Dissolve 1.00 g of copper (II) sulfate pentahydrate, 1.41 g of L-valine and 6.17 g of ammonium acetate in 800 mL of water, and add 200 mL of methanol.
Flow rate: Adjust so that the retention time of levofloxacin is about 20 minutes.
*System suitability—*
System performance: Dissolve 10 mg of ofloxacin in 20 mL of diluted 3 mol/L hydrochloric acid TS (1 in 100). To 1 mL of this solution add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, levofloxacin and an enantiomer 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 levofloxacin is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Levomepromazine Maleate

レボメプロマジンマレイン酸塩
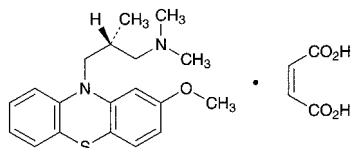
$C_{19}H_{24}N_2OS.C_4H_4O_4$: 444.54
(2*R*)-3-(2-Methoxy-10*H*-phenothiazin-10-yl)-
N,*N*,2-trimethylpropylamine monomaleate
[*7104-38-3*]

Levomepromazine Maleate, when dried, contains not less than 98.0% of levomepromazine maleate ($C_{19}H_{24}N_2OS.C_4H_4O_4$).

Description Levomepromazine Maleate occurs as white, crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

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

Melting point: 184 – 190°C (with decomposition).

Identification **(1)** Dissolve 5 mg of Levomepromazine Maleate in 5 mL of sulfuric acid: a red-purple color develops, which slowly becomes deep red-purple. To this solution add 1 drop of potassium dichromate TS: a brownish yellow-red color is produced.

(2) To 0.2 g of Levomepromazine Maleate add 5 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake well. Separate the diethyl ether layer, wash twice with 10-mL portions of water, add 0.5 g of anhydrous sodium sulfate, filter, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours: the residue melts <*2.60*> between 124°C and 128°C.

(3) To 0.5 g of Levomepromazine Maleate add 5 mL of water and 2 mL of ammonia solution (28), extract with three 5-mL portions of chloroform, separate and evaporate the water layer to dryness. To the residue add 2 to 3 drops of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine all the diethyl ether extracts, evaporate the diethyl ether in a water bath at a temperature of about 35°C with the aid of a current of air: the residue melts <*2.60*> between 128°C and 136°C.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: − 13.5 – − 16.5° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity **(1)** Clarity and color of solution—To 0.5 g of Levomepromazine Maleate add 10 mL of methanol, and dissolve by warming: the solution is clear, and colorless or pale yellow.

(2) Chloride <*1.03*>—Dissolve 0.5 g of Levomepromazine Maleate in 40 mL of methanol, 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 0.40 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Levomepromazine 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).

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 1 g of Levomepromazine Maleate, previously dried, and dissolve in a mixture of 40 mL of acetic acid (100) and 20 mL of acetone for nonaqueous titration. Titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red-purple through blue-purple to blue (indicator: 5 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.45 mg of $C_{19}H_{24}N_2OS.C_4H_4O_4$

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

Levothyroxine Sodium Hydrate

レボチロキシンナトリウム水和物
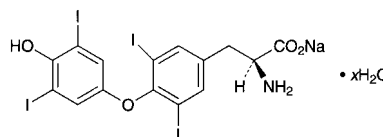
$C_{15}H_{10}I_4NNaO_4.xH_2O$
Monosodium *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosinate hydrate
[*25416-65-3*]

Levothyroxine Sodium Hydrate contains not less than 97.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85), calculated on the dried basis.

Description Levothyroxine Sodium Hydrate occurs as a pale yellowish white to light yellow-brown powder. It is odorless.

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

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Identification **(1)** Heat 0.1 g of Levothyroxine Sodium Hydrate over a flame: a purple gas evolves.

(2) To 0.5 mg of Levothyroxine Sodium Hydrate add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and add 0.1 mL of sodium nitrite TS. Allow to stand in a dark place for 20 minutes, and add 1.5 mL of ammonia solution (28): a yellowish red color is produced.

(3) Determine the absorption spectrum of a solution of Levothyroxine Sodium Hydrate in dilute sodium hydroxide TS (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.

(4) Moisten Levothyroxine Sodium Hydrate with sulfuric acid, and ignite: the residue responds to the Qualitative Tests <*1.09*> (1) and (2) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: − 5 – − 6° (0.3 g calculated on the dried basis, a mixture of ethanol (95) and sodium hydroxide TS (2:1), 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.3 g of Levothyroxine Sodium Hydrate in 10 mL of a mixture of ethanol (95) and sodium hydroxide TS (2:1) by warming: the solution is clear and pale yellow to pale yellow-brown in color.

(2) Soluble halides—Dissolve 0.01 g of Levothyroxine Sodium Hydrate in 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. To the filtrate add water to make 10 mL, then add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of water and 1 drop of dilute nitric acid, and proceed as directed above.

(3) Related substances—Dissolve 20 mg of Levothyroxine Sodium Hydrate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (14:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (14:1) 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 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 *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the red-purple 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 – 11% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Assay Weigh accurately about 25 mg of Levothyroxine Sodium Hydrate, and proceed as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of a freshly prepared sodium bisulfate solution (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.6657 mg of $C_{15}H_{10}I_4NNaO_4$

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

Levothyroxine Sodium Tablets

レボチロキシンナトリウム錠

Levothyroxine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85).

Method of preparation Prepare as directed under Tablets, with Levothyroxine Sodium Hydrate.

Identification **(1)** Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 0.5 mg of Levothyroxine Sodium Hydrate, add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and filter. To the filtrate add 0.1 mL of sodium nitrite TS, and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28): a yellowish red color develops.

(2) To a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 1 mg of Levothyroxine Sodium Hydrate, add 10 mL of ethanol (95), shake, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of levothyroxine sodium for thin-layer chromatography in 100 mL of ethanol (95), 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 *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same Rf value.

Purity Soluble halides—Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 2.5 mg of Levothyroxine Sodium Hydrate, add 25 mL of water, warm to 40°C, shake for 5 minutes, add 3 drops of dilute nitric acid, and filter. To the filtrate add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 25 mL of water and 3 drops of dilute nitric acid, and proceed as directed above.

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 Levothyroxine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, 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 calculate the ratio of the peak area of levothyroxine to that of the internal standard. Calculate the mean value from the ratios of each peak area of 10 samples: the deviation (%) of the mean value and the ratio of each peak area should be not more than 15%. When the deviation (%) is more than 15%, and 1 sam-

ple shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of the mean value of the 30 samples used in the 2 tests and the ratio of each peak area: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of ethinylestradiol in a mixture of acetonitrile and diluted phosphoric acid (1 in 10) (9:1) (3 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: a constant wavelength between 220 nm and 230 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

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

Mobile phase: A mixture of methanol, water and phosphoric acid (1340:660:1).

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

Selection of column: To 5 mL of a solution of levothyroxine sodium in 0.01 mol/L sodium hydroxide TS (1 in 200,000) add 1 mL of the internal standard solution. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of levothyroxine and the internal standard in this order with the resolution between these peaks being not less than 2.0.

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

Assay Weigh accurately and powder not less than 20 Levothyroxine Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 3 mg of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), into a crucible, and add potassium carbonate amounting to twice the mass of the powder. In the case that the weighed powder is less than 4 g, add 8 g of potassium carbonate to the crucible. Mix well, and gently tap the crucible on the bench to compact the mixture. Overlay with 10 g of potassium carbonate, and compact again by tapping. Heat the crucible strongly at a temperature between 675°C and 700°C for 25 minutes. Cool, add 30 mL of water, heat gently to boiling, and filter into a flask. To the residue add 30 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with hot water until the filtrate measures 300 mL. Add slowly 7 mL of freshly prepared bromine TS and diluted phosphoric acid (1 in 2) in the ratio of 3.5 mL to 1 g of the added potassium carbonate, and boil until starch-potassium iodide paper is no longer colored blue by the evolved gas. Wash the inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume of not less than 250 mL. Cool, add 5 mL of a solution of phenol (1 in 20), again rinse the inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate <2.50> immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.
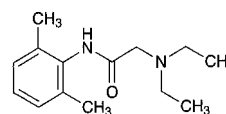
<div align="center">

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.3329 mg of $C_{15}H_{10}I_4NNaO_4$

</div>

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

Lidocaine

リドカイン

$C_{14}H_{22}N_2O$: 234.34
2-Diethylamino-*N*-(2,6-dimethylphenyl)acetamide
[*137-58-6*]

Lidocaine, when dried, contains not less than 99.0% of lidocaine ($C_{14}H_{22}N_2O$).

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

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

It dissolves in dilute hydrochloric acid.

Identification **(1)** Dissolve 40 mg of Lidocaine in 10 mL of 1 mol/L hydrochloric acid TS, and 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.

(2) Determine the infrared absorption spectrum of Lidocaine 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> 66 – 69°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Lidocaine in 2 mL of dilute hydrochloric acid, and add water to make 10 mL: the solution is clear and colorless to light yellow.

(2) Chloride <1.03>—Dissolve 0.6 g of Lidocaine in 6 mL of dilute nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

(3) Sulfate <1.14>—Dissolve 0.5 g of Lidocaine in 5 mL of dilute hydrochloric acid, add water to make 50 mL, and 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, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.096%).

(4) Heavy metals <1.07>—Carbonize 2.0 g of Lidocaine by gentle ignition. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed 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) Related substances—Dissolve 0.10 g of Lidocaine in 2 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 chromatog-

raphy. Develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, 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, 24 hours).

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

Assay   Dissolve about 0.5 g of Lidocaine, previously dried and accurately weighed, in 20 mL of acetic acid (100), and titrate *<2.50>* with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS) until the color of the solution changes from purple to blue-green through blue. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.43 mg of $C_{14}H_{22}N_2O$

Containers and storage   Containers—Tight containers.

Lidocaine Injection

Lidocaine Hydrochloride Injection

リドカイン注射液

Lidocaine Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O.HCl$: 270.80).

Method of preparation   Prepare as directed under Injections, with Lidocaine and an equivalent amount of Hydrochloric Acid.

No preservative is added in the case of intravenous injections.

Description   Lidocaine Injection is a colorless, clear liquid.
pH: 5.0 – 7.0

Identification   To a volume of Lidocaine Injection, equivalent to 20 mg of lidocaine hydrochloride ($C_{14}H_{22}N_2O.HCl$), add 1 mL of sodium hydroxide TS, and extract with 20 mL of hexane. To 10 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, and shake vigorously. Determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 261 nm and 265 nm.

Bacterial endotoxins *<4.01>*   Less than 1.0 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 exactly measured volume of Lidocaine Injection, equivalent to about 0.1 g of lidocaine hydrochloride ($C_{14}H_{22}N_2O.HCl$), add exactly 10 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Sepa-

rately, weigh accurately about 85 mg of lidocaine for assay, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in 0.5 mL of 1 mol/L hydrochloric acid TS and a suitable volume of 0.001 mol/L hydrochloric acid TS, and add exactly 10 mL of the internal standard solution, then add 0.001 mol/L hydrochloric acid TS 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 lidocaine to that of the internal standard.

Amount (mg) of lidocaine hydrochloride
($C_{14}H_{22}N_2O.HCl$)
$= M_S \times Q_T/Q_S \times 1.156$

M_S: Amount (mg) of lidocaine for assay taken

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).
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 (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution (pH 3.0) and acetonitrile (11:9).
Flow rate: Adjust so that the retention time of lidocaine is about 6 minutes.
*System suitability*—
System performance: When proceed with 5 μL of the standard solution under the above operating conditions, lidocaine 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of lidocaine to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Hermetic containers.

Limaprost Alfadex

リマプロスト　アルファデクス
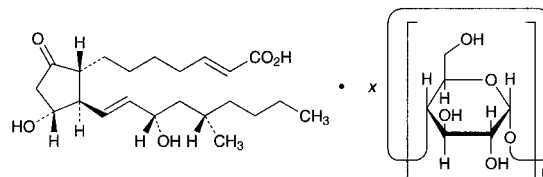
$C_{22}H_{36}O_5 \cdot xC_{36}H_{60}O_{30}$
(2*E*)-7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*,5*S*)-3-hydroxy-5-methylnon-1-en-1-yl]-5-oxocyclopentyl}hept-2-enoic acid-α-cyclodextrin
[*100459-01-6*, limaprost:alfadex = 1:1; clathrate compound]

Limaprost Alfadex is a α-cyclodextrin clathrate compound of limaprost.

It contains not less than 2.8% and not more than 3.2% of limaprost ($C_{22}H_{36}O_5$: 380.52), calculated on

the anhydrous basis.

Description Limaprost Alfadex occurs as a white powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in ethyl acetate.

It is hygroscopic.

Identification **(1)** Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and use the upper layer as the sample solution (1). Separately, to 20 mg of Limaprost Alfadex add 5 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution (2). Evaporate the solvent of the sample solutions (1) and (2) under reduced pressure, add 2 mL of sulfuric acid to each of the residue, and shake them for 5 minutes: the solution obtained from the sample solution (1) develops an orange-yellow color while the solution obtained from the sample solution (2) does not develop any color.

(2) Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent of the upper layer under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), 5 mL of 1,3-dinitrobenzene TS, add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) while ice-cooling, and allow to stand in a dark place while ice-cooling for 20 minutes: a purple color develops.

(3) To 50 mg of Limaprost Alfadex add 1 mL of iodine TS, dissolve by heating in a water bath, and allow to stand: a dark blue precipitate is formed.

(4) Determine the absorption spectrum of a solution of Limaprost Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it does not exhibit a maximum between 200 nm and 400 nm. To 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes. 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.

Optical rotation <2.49> $[\alpha]_D^{20}$: +125 – 135° (0.1 g calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

Purity Related substances—Perform the test immediately after preparation of the sample solution. Dissolve 0.10 g of Limaprost Alfadex in 2 mL of water, add 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 3 mL of the standard solution (1), add dilute ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 3 μL each of the sample solution and standard solutions (1) and (2) 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 of 17-epi-isomer, having the relative retention time of about 1.1 to limaprost, and the area of the peak of 11-deoxy substance, having the relative retention time of about 2.1, are not larger than the peak area of limaprost from the standard solution (2), and the area of the peak other than the principal peak and the peaks mentioned above is not larger than 1/3 times the peak area of limaprost from the standard solution (2). The total area of the peaks other than limaprost from the samples solution is not larger than the peak area of limaprost from the standard solution (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 3 times as long as the retention time of limaprost 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 standard solution (1) add dilute ethanol to make exactly 10 mL. Confirm that the peak area of limaprost obtained from 3 μL of this solution is equivalent to 8 to 12% of that obtained from 3 μL of the standard solution (1).

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution (1) under the above conditions, the relative standard deviation of the peak area of limaprost is not more than 2.0%.

Water <2.48> Not more than 6.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Limaprost Afladex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Limaprost RS, dissolve in exactly 5 mL of the internal standard solution, add 5 mL of water, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and the 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 limaprost to that of the internal standard.

$$\text{Amount (mg) of limaprost } (C_{22}H_{36}O_5) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Limaprost RS taken

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

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: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, acetonitrile for liquid chromatography and 2-propanol for liquid chromatography (9:5:2).

Flow rate: Adjust so that the retention time of limaprost 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, the internal standard and limaprost 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 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of limaprost to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding −10°C.

Lincomycin Hydrochloride Hydrate

リンコマイシン塩酸塩水和物
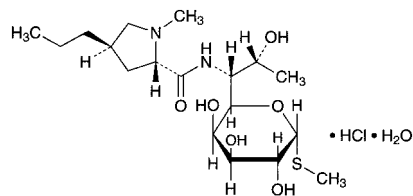
$C_{18}H_{34}N_2O_6S.HCl.H_2O$: 461.01
Methyl 6,8-dideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside monohydrochloride monohydrate
[*7179-49-9*]

Lincomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis*.

It contains not less than 850 µg (potency) and not more than 930 µg (potency) per mg, calculated on the anhydrous basis. The potency of Lincomycin Hydrochloride Hydrate is expressed as mass (potency) of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Description   Lincomycin Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

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

Identification   **(1)**   Determine the infrared absorption spectrum of Lincomycin Hydrochloride Hydrate as directed in the paste method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum or the spectrum of Lincomycin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

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

Optical rotation *<2.49>* $[\alpha]_D^{20}$: +135 – +150° (0.5 g, water, 25 mL, 100 mm).

pH *<2.54>*   Dissolve 0.10 g of Lincomycin Hydrochloride Hydrate in 1 mL of water: 3.0 – 5.5.

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

(2)   Heavy metals *<1.07>*—Proceed with 2.0 g of Lincomycin Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3)   Lincomycin B—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 determine the peak areas of lincomycin and lincomycin B, having the relative retention time of about 0.5 to lincomycin obtained from the sample solution, by the automatic integration method: the peak area of lincomycin B is not more than 2.0% of the sum of the peak areas of lincomycin and lincomycin B.

Operating conditions—
Proceed as directed in the operating conditions in the Assay.

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

Test for required detectability: Measure exactly 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of lincomycin obtained from 20 µL of this solution is equivalent to 1.4 to 2.6% of that obtained from 20 µL of the sample solution.

Water *<2.48>*   3.0 – 6.0% (0.5 g, volumetric titration, direct titration).

Assay   Weigh accurately an amount of Lincomycin Hydrochloride Hydrate and Lincomycin Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and 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 lincomycin in each solution.

Amount [µg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$)
$= M_S \times A_T/A_S \times 1000$

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

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 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 46°C.

Mobile phase: To 13.5 mL phosphoric acid add 1000 mL of water, and adjust the pH to 6.0 with ammonia TS. To 780 mL of this solution add 150 mL of acetonitrile and 150 mL of methanol.

Flow rate: Adjust so that the retention time of lincomycin 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 lincomycin are not less than 4000 and not more than 1.3, 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 lincomycin is not more than 2.0%.

Containers and storage   Containers—Tight containers.

Lincomycin Hydrochloride Injection

リンコマイシン塩酸塩注射液

Lincomycin Hydrochloride Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Method of preparation   Prepare as directed under Injections, with Lincomycin Hydrochloride Hydrate.

Description   Lincomycin Hydrochloride Injection is a clear, colorless liquid.

Identification   To a volume of Lincomycin Hydrochloride Injection, equivalent to 30 mg (potency) of Lincomycin Hydrochloride Hydrate, add 30 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Lincomycin Hydrochloride RS 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 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Dissolve 150 g of ammonium acetate in 800 mL of water, adjust the pH to 9.6 with ammonia solution (28), and add water to make 1000 mL. To 80 mL of this solution add 40 mL of 2-propanol and 90 mL of ethyl acetate, shake, develop the plate with the upper layer of this solution to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of potassium permanganate (1 in 1000) on the plate: the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same Rf value.

pH <2.54>   3.5 – 5.5

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 Lincomycin Hydrochloride Injection, equivalent to about 0.3 g (potency) of Lincomycin Hydrochloride Hydrate, add the mobile phase to make exactly 30 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lincomycin Hydrochloride RS, equivalent to 20 mg (potency), dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Lincomycin Hydrochloride Hydrate.
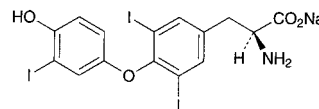
Amount [mg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$)
 $= M_S \times A_T/A_S \times 15$

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

Containers and storage   Containers—Hermetic containers.

Liothyronine Sodium

リオチロニンナトリウム

$C_{15}H_{11}I_3NNaO_4$: 672.96
Monosodium *O*-(4-hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosinate
[*55-06-1*]

Liothyronine Sodium contains not less than 95.0% of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$), calculated on the dried basis.

Description   Liothyronine Sodium occurs as a white to light brown powder. It is odorless.
 It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.
 It dissolves in sodium hydroxide TS and in ammonia TS.

Identification   **(1)**   To 5 mL of a solution of Liothyronine Sodium in ethanol (95) (1 in 1000) add 1 mL of ninhydrin TS, and warm in a water bath for 5 minutes: a purple color develops.
 (2)   Heat 0.02 g of Liothyronine Sodium with a few drops of sulfuric acid over a flame: a purple gas is evolved.
 (3)   Determine the absorption spectrum of a solution of Liothyronine Sodium in ethanol (95) (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.
 (4)   Ignite 0.02 g of Liothyronine Sodium until thoroughly charred. After cooling, add 5 mL of water to the residue, shake, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+18$ – $+22°$ (0.2 g calculated on the dried basis, a mixture of ethanol (95) and 1 mol/L hydrochloric acid TS (4:1), 10 mL, 100 mm).

Purity   **(1)**   Soluble halide—To 10 mg of Liothyronine Sodium add 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. Add water to the filtrate to make 10 mL, and mix with 3 drops of silver nitrate TS: the solution shows no more turbidity than the following control solution.
 Control solution: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 1 drop of dilute nitric acid and water to make 10 mL, and add 3 drops of silver nitrate TS.
 (2)   Iodine and iodide—Dissolve 0.10 g of Liothyronine Sodium in 10 mL of dilute sodium hydroxide TS and 15 mL of water, add 5 mL of dilute sulfuric acid, and allow to stand for 10 minutes with occasional shaking. Filter the mixture into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100) to the filtrate, mix for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.
 Control solution: Weigh exactly 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. Pipet 1 mL of this solution, add 10 mL of dilute hydroxide TS, 14 mL of water and 5 mL of dilute sulfuric acid, and mix. Filter the mixture into a Nessler tube, and perform the test with the filtrate in the same manner as for the sample.

(3)   Related substances—Dissolve 0.15 g of Liothyronine Sodium in 5 mL of diluted ammonia TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ammonia TS (1 in 3) 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 *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 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 4.0% (0.2 g, 105°C, 2 hours).

Assay   Weigh accurately about 25 mg of Liothyronine Sodium, and proceed as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 10 mL of a solution of sodium hydroxide (1 in 100) and 1 mL of a freshly prepared solution of sodium bisulfate (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water again. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

<div align="center">

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.7477 mg of $C_{15}H_{11}I_3NNaO_4$

</div>

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

Liothyronine Sodium Tablets

リオチロニンナトリウム錠

Liothyronine Sodium Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$: 672.96).

Method of preparation   Prepare as directed under Tablets, with Liothyronine Sodium.

Identification   (1)   To a glass-stoppered centrifuge tube add a portion of finely powdered Liothyronine Sodium Tablets, equivalent to 0.1 mg of Liothyronine Sodium, add 30 mL of dilute sodium hydroxide TS, shake vigorously, and centrifuge. Transfer the supernatant liquid to a separator,

add 10 mL of dilute hydrochloric acid, and extract with two 20-mL portions of ethyl acetate. Filter each extract successively through absorbent cotton previously overlaid with 8 g of anhydrous sodium sulfate. Evaporate the filtrate on a water bath to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of liothyronine sodium for thin-layer chromatography in 50 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 *t*-butyl alcohol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same *R*f value.

(2)   The colored solution obtained in the Assay is blue in color.

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 Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge for 5 minutes, and filter the supernatant liquid, if necessary. Pipet a definite volume of this solution, and add a volume of 0.01 mol/L sodium hydroxide TS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) per mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution 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, and calculate the ratio of the peak area of the liothyronine to that of the internal standard. Calculate the mean value of the ratios of each peak area of 10 samples: the deviation (%) of each ratio of the peak area from the mean value should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of each ratio of the peak area from the mean value of the 30 samples used in the two tests: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of propylpara-hydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9:1) (1 in 250,000).

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 octadecylsylanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Diluted methanol (57 in 100).

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

System suitability—

System performance: To 5 mL of a solution of liothyronine sodium in 0.01 mol/L sodium hydroxide TS (1 in 2,000,000) add 1 mL of the internal standard solution, and use this solution as the solution for system suitability test. When the procedure is run with 200 μL of this solution under the above operating conditions, the internal standard and liothyronine 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of liothyronine to that of the internal standard is not more than 1.0%.

Assay   Weigh accurately not less than 20 Liothyronine Sodium Tablets, and finely powder. Place an accurately weighed portion of the powder, equivalent to about 50 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$), in an agate mortar, add 1 g of powdered potassium carbonate, and mix well. Transfer the mixture cautiously to a porcelain crucible, and compact the contents by gently tapping the crucible on a table. Add an additional 1.5 g of powdered potassium carbonate to the same agate mortar, mix well with any content adhering to the mortar, cautiously overlay the mixture on the top of the same porcelain crucible, and compact the charge again in the same manner. Ignite the combined mixture in the crucible between 675°C and 700°C for 30 minutes. Cool, add a few mL of water to the crucible, heat gently to boiling, and filter the contents of the crucible through a glass filter (G4) into a 20-mL volumetric flask. Wash the residue with water, and combine the washings with the filtrate. Cool, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 200 mL. Measure exactly 5 mL of the solution, and add a solution of potassium carbonate (1 in 8) to make exactly 100 mL. To 2 mL of this solution, exactly measured, add a solution of potassium carbonate (1 in 8) to make exactly 20 mL, and use the solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into glass-stoppered test tubes, add 3.0 mL of diluted sulfuric acid (4 in 25) and 2.0 mL of potassium permanganate TS, and heat on a water bath for 15 minutes. Cool, add 1.0 mL of diluted sodium nitrite TS (1 in 10), swirl to mix, and add 1.0 mL of a solution of ammonium amidosulfate (1 in 10). Allow to stand at room temperature for 10 minutes with occasional shaking. Then add 1.0 mL of potato starch TS and 1.0 mL of a freshly prepared, diluted potassium iodide TS (1 in 40), swirl to mix, and transfer each solution to a 20-mL volumetric flask. Rinse the test tube with water, collect the washings in the volumetric flask, add water to make 20 mL, and allow to stand for 10 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of potassium carbonate (1 in 8) in the same manner as the sample solution as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at the wavelength of maximum absorption at about 600 nm, respectively.
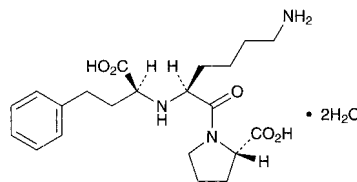
Amount (mg) of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$)
$= M_S \times A_T/A_S \times 1/2000 \times 1.351$

M_S: Amount (mg) of potassium iodide for assay taken

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

Lisinopril Hydrate

リシノプリル水和物

$C_{21}H_{31}N_3O_5 \cdot 2H_2O$: 441.52
(2S)-1-{(2S)-6-Amino-2-[(1S)-1-carboxy-3-phenylpropylamino]hexanoyl}pyrrolidine-2-carboxylic acid dihydrate
[*83915-83-7*]

Lisinopril Hydrate contains not less than 98.5% and not more than 101.0% of lisinopril ($C_{21}H_{31}N_3O_5$: 405.49), calculated on the anhydrous basis.

Description   Lisinopril Hydrate occurs as a white crystalline powder, having a slight characteristic odor.

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

Melting point: about 160°C (with decomposition).

Identification   **(1)**   Determine the absorption spectrum of a solution of Lisinopril Hydrate in methanol (1 in 1000) 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 Lisinopril Hydrate 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.

Optical rotation <2.49> $[\alpha]_D^{25}$: −43.0 – −47.0° (0.25 g calculated on the anhydrous basis, 0.25 mol/L zinc acetate buffer solution (pH 6.4), 25 mL, 100 mm).

Purity   **(1)**   Heavy metals <1.07>—Proceed with 2.0 g of Lisinopril 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 about 0.10 g of Lisinopril Hydrate in 50 mL of water, and use this solution as the sample solution. Pipet 3 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 15 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 1.2 to lisinopril from the sample solution, is not larger than 1/5 times the peak area of lisinopril from the standard solution, the area of the peak other than lisinopril and the peak mentioned above is not larger than 2/15 times the peak area of lisinopril from the standard solution, and the total area of the peaks other than lisinopril is not larger than the peak area of lisinopril from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.0 mm in inside diame-

ter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

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

Mobile phase A: Diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2).

Mobile phase B: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (3:2).

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 → 50	10 → 50
10 – 25	50	50

Flow rate: About 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of lisinopril, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2.5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 15 μL of the standard solution.

System performance: To 10 mg of lisinopril hydrate and 2 mL of a solution of anhydrous caffeine (1 in 1000) add water to make 200 mL. When the procedure is run with 15 μL of this solution under the above operating conditions, lisinopril and caffeine 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 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Water *<2.48>*   Not less than 8.0% and not more than 9.5% (0.3 g, volumetric titration, back titration).

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

Assay   Weigh accurately about 0.66 g of Lisinopril Hydrate, dissolve in 80 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
= 40.55 mg of $C_{21}H_{31}N_3O_5$

Containers and storage   Containers—Well-closed containers.

Lisinopril Tablets

リシノプリル錠

Lisinopril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$: 405.49).

Method of preparation   Prepare as directed under Tablets, with Lisinopril Hydrate.

Identification   To an amount of powdered Lisinopril Tablets, equivalent to 10 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of lisinopril 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 30 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, acetic acid (100), water and ethyl acetate (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 120°C: the principal spot with the sample solution and the spot with the standard solution show a red-purple color and their *R*f values are the same.

Purity   Related substances—Powder not less than 20 Lisinopril Tablets. Take a portion of the powder, equivalent to about 25 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add 25 mL of water, shake for 20 minutes, filter, and use the filtrate as the sample solution. Pipet 3 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 15 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 lisinopril diketopiperazine, having the relative retention time of about 2.0 to lisinopril from the sample solution, is not larger than 2/3 times the peak area of lisinopril from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2) under Lisinopril Hydrate.

System suitability—

System performance: Proceed as directed in the system suitability in the Purity (2) under Lisinopril Hydrate.

Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 15 μL of the standard solution.

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 lisinopril 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 Lisinopril Tablets add exactly 5 mL of the internal standard solution per 1 mg of lisinopril ($C_{21}H_{31}N_3O_5$), shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Hereafter, proceed as directed in the Assay.

Amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$)
$$= M_S \times Q_T/Q_S \times C/10$$

M_S: Amount (mg) of lisinopril for assay taken, calculated on the anhydrous basis
C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

Internal standard solution—A solution of anhydrous caffeine (1 in 20,000).

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 of a 5-mg tablet in 60 minutes and that of a 10-mg tablet in 90 minutes is not less than 80%, and that of a 20-mg tablet

in 90 minutes is not less than 75%.

Start the test with 1 tablet of Lisinopril 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 5.6 μg of lisinopril ($C_{21}H_{31}N_3O_5$), and use this solution as the sample solution. Separately, weigh accurately about 15 mg of lisinopril for assay (separately determined the water <2.48> in the same manner as Lisinopril Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 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 lisinopril in each solution.

Dissolution rate (%) with respect to the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$$

M_S: Amount (mg) of lisinopril for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

Operating conditions—

Detector, column temperature, and mobile phase: 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).

Flow rate: Adjust so that the retention time of lisinopril 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 lisinopril are not less than 1000 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 lisinopril is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Lisinopril Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add exactly 25 mL of the internal standard solution, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of lisinopril for assay (separately determined the water <2.48> in the same manner as Lisinopril Hydrate), add exactly 50 mL of the internal standard solution to dissolve, 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 lisinopril to that of the internal standard.

Amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$)
$$= M_S \times Q_T/Q_S \times 1/2$$

M_S: Amount (mg) of lisinopril for assay taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of anhydrous

caffeine (1 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

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

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (19:1).

Flow rate: Adjust so that the retention time of lisinopril 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, lisinopril 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of lisinopril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Lithium Carbonate

炭酸リチウム

Li$_2$CO$_3$: 73.89

Lithium Carbonate, when dried, contains not less than 99.5% of lithium carbonate (Li$_2$CO$_3$).

Description Lithium Carbonate occurs as a white crystalline powder. It is odorless.

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

It dissolves in dilute acetic acid.

The pH of a solution dissolved 1.0 g of Lithium Carbonate in 100 mL of water is between 10.9 and 11.5.

Identification (1) Perform the test as directed under Flame Coloration Test <1.04> (1) with Lithium Carbonate: a persistent red color appears.

(2) Dissolve 0.2 g of Lithium Carbonate in 3 mL of dilute hydrochloric acid, and add 4 mL of sodium hydroxide TS and 2 mL of disodium hydrogen phosphate TS: a white precipitate is produced. To the precipitate add 2 mL of dilute hydrochloric acid: it dissolves.

(3) A solution of Lithium Carbonate (1 in 100) responds to the Qualitative Tests <1.09> for carbonate.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Lithium Carbonate in 10 mL of water by warming: the solution is clear and colorless.

(2) Acetic acid-insoluble substances—Take 1.0 g of Lithium Carbonate, dissolve in 40 mL of dilute acetic acid, filter the insoluble substances using filter paper for quantitative analysis, wash with five 10-mL portions of water, and ignite the insoluble substances together with the filter paper to incinerate: the mass of the residue is not more than 1.5

mg.

(3) Chloride <1.03>—To 0.40 g of Lithium Carbonate add 10 mL of water and 7 mL of dilute nitric acid, and dissolve by heating to boil. After cooling, 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 with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

(4) Sulfate <1.14>—To 0.40 g of Lithium Carbonate add 10 mL of water and 4 mL of dilute hydrochloric acid, and dissolve by heating to boil. After cooling, add 1 mL of dilute hydrochloric acid, and dilute with 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%).

(5) Heavy metals <1.07>—To 4.0 g of Lithium Carbonate add 5 mL of water, gradually add 10 mL of hydrochloric acid while mixing, and dissolve. Evaporate the solution on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a slight red color, then add 2 mL of dilute acetic 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: Evaporate 10 mL of hydrochloric acid on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a pale red color, then add 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to make 50 mL (not more than 5 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of Lithium Carbonate according to Method 2 using 11 mL of dilute hydrochloric acid, and perform the test according to Method B. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Aluminum—To 5.0 g of Lithium Carbonate add 20 mL of water, add gradually 15 mL of hydrochloric acid while stirring, and evaporate to dryness on a water bath. To the residue add 50 mL of water to dissolve, filter if necessary, and assign this solution as solution A. Separately, evaporate 15 mL of hydrochloric acid to dryness on a water bath, then proceed in the same manner, and assign the solution so obtained as solution B. To 10 mL of solution A add 10 mL of water and 5 mL of acetic acid-sodium acetate buffer solution (pH 4.5), and shake. Add 1 mL of a solution of L-ascorbic acid (1 in 100), 2 mL of aluminon TS and water to make 50 mL, shake well, and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 0.1758 g of aluminum potassium sulfate dodecahydrate in water to make 1000 mL. To 1.0 mL of this solution add 10 mL of solution B and water to make 20 mL, add 5 mL of acetic acid-sodium acetate buffer solution (pH 4.5), and proceed in the same manner.

(8) Barium—To 20 mL of solution A obtained in (7) add 6 mL of water, 0.5 mL of dilute hydrochloric acid, 3 mL of ethanol (95) and 2 mL of potassium sulfate TS, and allow to stand for 1 hour: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 17.8 mg of barium chloride dihydrate in water to make 1000 mL. To 6 mL of this solution add 20 mL of solution B obtained in (7), 0.5 mL of dilute hydrochloric acid and 3 mL of ethanol (95), and proceed in the same manner.

(9) Calcium—Weigh accurately about 5 g of Lithium Carbonate, add 50 mL of water and 15 mL of hydrochloric acid, and dissolve. Remove carbon dioxide from the solution by boiling, add 5 mL of ammonium oxalate TS, then make alkaline with ammonia TS, and allow to stand for 4 hours. Filter the produced precipitate through a glass filter (G4), wash with warm water until the turbidity of the washing is not produced with calcium chloride TS within 1 minute. Transfer the precipitate and the glass filter into a beaker, add water until the glass filter is covered with water, then add 3 mL of sulfuric acid, heat between 70°C and 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS until a pale red color persists for 30 seconds: the amount of calcium (Ca: 40.08) is not more than 0.05%.

Each mL of 0.02 mol/L potassium permanganate VS
= 2.004 mg of Ca

(10) Magnesium—To 3.0 mL of solution A obtained in (7) add 0.2 mL of a solution of titan yellow (1 in 1000) and water to make 20 mL, then add 5 mL of sodium hydroxide (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To 6 mL of this solution add 3 mL of solution B obtained in (7), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

(11) Potassium—Dissolve 1.0 g of Lithium Carbonate in water to make 100 mL, and use this solution as the sample solution. To 5 mL of the sample solution add 1.0 mL of dilute acetic acid, shake, add 5 mL of a solution of sodium tetraphenylborate (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water to make 1000 mL. To 5 mL of this solution add 1.0 mL of dilute acetic acid, shake, and proceed in the same manner.

(12) Sodium—Weigh accurately about 0.8 g of Lithium Carbonate, dissolve in water to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly 25 mL of the sample stock solution, add water to make exactly 100 mL, and use this solution as the sample solution (1). Separately, weigh accurately 25.4 mg of sodium chloride, dissolve in water to make exactly 1000 mL, and use this solution as the standard solution. Measure exactly 25 mL of the sample stock solution, add exactly 20 mL of the standard solution, then add water to make exactly 100 mL, and use this solution as the sample solution (2). Determine emission intensities of sodium using a flame photometer with the sample solution (1) and the sample solution (2) under the following conditions. Adjust the wavelength dial to 589 nm, atomize the sample solution (2) into the flame, then adjust the sensitivity so that the emission intensity L_S shows 100 adjustment, and determine emission intensity L_T of the sample solution (1). Then, make the other conditions identical, change the wavelength dial to 580 nm, determine emission intensity L_B of the sample solution (1): the amount of sodium, calculated from the following equation, is not more than 0.05%.

Amount (%) of sodium (Na)
$$= (L_T - L_B)/(L_S - L_T) \times M'/M \times 100$$

M: Amount (mg) of the sample in 25 mL of the sample stock solution

M': Amount (mg) of sodium in 20 mL of the standard solution

(13) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Lithium Carbonate, add 2 mL of water and 3 mL of hydrochloric acid, and perform the test (not more than 2 ppm).

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

Assay Weigh accurately about 1 g of Lithium Carbonate, previously dried, add exactly 100 mL of water and 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool, and titrate <*2.50*> the excess sulfuric acid with 1 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

$$\text{Each mL of 0.5 mol/L sulfuric acid VS}$$
$$= 36.95 \text{ mg of Li}_2\text{CO}_3$$

Containers and storage Containers—Well-closed containers.

Lobenzarit Sodium

ロベンザリットナトリウム
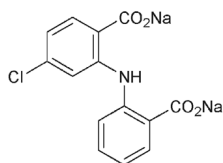
C$_{14}$H$_8$ClNNa$_2$O$_4$: 335.65
Disodium 2-[(2-carboxylatophenyl)amino]-4-chlorobenzoate
[*64808-48-6*]

Lobenzarit Sodium, when dried, contains not less than 98.0% and not more than 101.0% of lobenzarit sodium (C$_{14}$H$_8$ClNNa$_2$O$_4$).

Description Lobenzarit Sodium occurs as a white to pale yellowish white crystalline powder.

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

Identification **(1)** A solution of Lobenzarit Sodium (1 in 50) responds to the Qualitative Tests <*1.09*> (1) for chloride.

(2) Determine the absorption spectrum of a solution of Lobenzarit Sodium (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 Lobenzarit 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 Lobenzarit Sodium (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for sodium salt.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Lobenzarit 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).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Lobenzarit Sodium according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 50 mg of Lobenzarit Sodium in 2.5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add 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 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 tetrahydrofuran, water and triethylamine (50:15:8) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): 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 1.0% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 0.1 g of Lobenzarit Sodium, previously dried, dissolve in exactly 40 mL of water, add exactly 60 mL of a mixture of diethyl ether and tetrahydrofuran (1:1), and titrate <*2.50*> with 0.1 mol/L hydrochloric acid VS while well shaking (indicator: 10 drops of bromophenol blue TS) until the blue color of the water layer changes to a persistent light blue-green. Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L hydrochloric acid VS}$$
$$= 16.78 \text{ mg of C}_{14}\text{H}_8\text{ClNNa}_2\text{O}_4$$

Containers and storage Containers—Tight containers.

Lorazepam

ロラゼパム
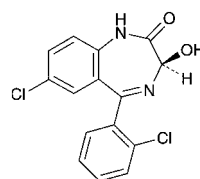
and enantiomer

C$_{15}$H$_{10}$Cl$_2$N$_2$O$_2$: 321.16
(3*RS*)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*846-49-1*]

Lorazepam, when dried, contains not less than 98.5% of lorazepam (C$_{15}$H$_{10}$Cl$_2$N$_2$O$_2$).

Description Lorazepam occurs as a white crystalline powder. It is odorless.

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

It is gradually colored by light.

Identification **(1)** To 0.02 g of Lorazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, and cool: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(2) Determine the absorption spectrum of a solution of Lorazepam 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.

(3) Determine the infrared absorption spectrum of

Lorazepam, 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 Lorazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Absorbance <2.24> $E_{1\,cm}^{1\%}$ (229 nm): 1080 – 1126 (after drying, 1 mg, ethanol (95), 200 mL).

Purity (1) Chloride <1.03>—To 1.0 g of Lorazepam 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%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Lorazepam 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 Lorazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Lorazepam in 20 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 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, 1,4-dioxane and acetic acid (100) (91:5:4) 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, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.4 g of Lorazepam, previously dried, dissolve in 50 mL of acetone, and titrate <2.50> with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
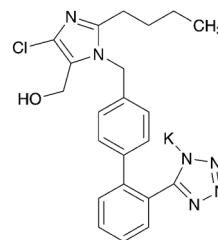
> Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS
>   = 32.12 mg of $C_{15}H_{10}Cl_2N_2O_2$

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

Losartan Potassium

ロサルタンカリウム

$C_{22}H_{22}ClKN_6O$: 461.00
Monopotassium 5-{[4′-(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl}-1*H*-tetrazol-1-ide
[*124750-99-8*]

Losartan Potassium contains not less than 98.5% and not more than 101.0% of losartan potassium ($C_{22}H_{22}ClKN_6O$), calculated on the anhydrous basis.

Description Losartan Potassium occurs as a white crystalline powder.
  It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Losartan Potassium 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 Losartan Potassium 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 Losartan Potassium 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 Losartan Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Losartan Potassium responds to the Qualitative Tests <1.09> (1) for potassium salt.

(4) Perform the test with Losartan Potassium as directed under Flame Coloration Test <1.04> (2): a green color appears.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Losartan Potassium 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 Losartan Potassium in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol 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 each peak other than the peaks of solvent and losartan obtained from the sample solution is not larger than 1/10 times the peak area of losartan obtained from the standard solution, and the total area of the peaks other than losartan from the sample solution is not larger than 3/10 times the peak area of losartan from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 25°C.

Mobile phase A: Diluted phosphoric acid (1 in 1000).

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 – 25	75 → 10	25 → 90
25 – 35	10	90

Flow rate: 1.0 mL per minute.

Time span of measurement: For 35 minutes after injection of the sample solution.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of losartan obtained from 10 μL of this solution is equivalent to 7 to 13% of that of losartan 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 losartan are not less than 10,000 and not more than 1.3, 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 losartan is not more than 2.0%.

Water <2.48> Not more than 0.5% (0.25 g, volumetric titration, direct titration).

Assay Weigh accurately about 25 mg each of Losartan Potassium and Losartan Potassium RS (separately, determine the water <2.48> in the same manner as Losartan Potassium), dissolve separately in methanol 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 losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$)
= $M_S \times A_T/A_S$

M_S: Amount (mg) of Losartan Potassium RS taken, calculated on the anhydrous basis

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 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 diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of losartan 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 losartan are not less than 5500 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 losartan is not more than 1.0%.

Containers and storage Containers—Tight containers.

Losartan Potassium Tablets

ロサルタンカリウム錠

Losartan Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$: 461.00).

Method of preparation Prepare as directed under Tablets, with Losartan Potassium.

Identification To an amount of powdered Losartan Potassium Tablets, equivalent to 25 mg of losartan potassium, add 10 mL of methanol, shake well, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 25 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of losartan potassium in 10 mL of methanol. To 5 mL of this solution add methanol to make 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 ethyl acetate, methanol and acetic acid (100) (75:25:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same Rf 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.

To 1 tablet of Losartan Potassium Tablets add diluted 0.1 mol/L phosphate buffer solution (pH 8.0) (1 in 10) to make exactly 100 mL, and stir until the tablet is completely disintegrated. Pipet 5 mL of this solution, add diluted 0.1 mol/L phosphate buffer solution (pH 8.0) (1 in 10) to make exactly V mL so that each mL contains about 50 μg of losartan potassium ($C_{22}H_{22}ClKN_6O$), centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$)
= $M_S \times A_T/A_S \times V/25$

M_S: Amount (mg) of Losartan Potassium RS taken, calculated on the anhydrous basis

Dissolution <6.10> When the test is performed at 50 revolutions per minute for 25-mg and 50-mg tablets and at 75 revolutions per minute for 100-mg tablet according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of 25-mg and 50-mg tablets, and in 30 minutes of 100-mg tablet are not less than

85%, respectively.

Start the test with 1 tablet of Losartan Potassium 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 water to make exactly V' mL so that each mL contains about 22 μg of losartan potassium ($C_{22}H_{22}ClKN_6O$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Losartan Potassium RS (separately determine the water <2.48> in the same manner as Losartan Potassium), 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, at 256 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 losartan potassium ($C_{22}H_{22}ClKN_6O$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of Losartan Potassium RS taken, calculated on the anhydrous basis

C: Labeled amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) in 1 tablet

Assay To 20 Losartan Potassium Tablets add diluted 0.1 mol/L phosphate buffer solution (pH 8.0) (1 in 10) to make exactly 1000 mL, and stir until the tablets are completely disintegrated. Pipet 5 mL of this solution, add diluted 0.1 mol/L phosphate buffer solution (pH 8.0) (1 in 10) to make exactly V mL so that each mL contains about 50 μg of losartan potassium ($C_{22}H_{22}ClKN_6O$), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Losartan Potassium RS (separately determine the water <2.48> in the same manner as Losartan Potassium), dissolve in diluted 0.1 mol/L phosphate buffer solution (pH 8.0) (1 in 10) to make exactly 500 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 losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) in 1 tablet
$$= M_S \times A_T/A_S \times V/50$$

M_S: Amount (mg) of Losartan Potassium RS taken, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 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 35°C.
Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 2.5 with phosphoric acid, and add water to make 1000 mL. To 600 mL of this solution add 400 mL of acetonitrile
Flow rate: Adjust so that the retention time of losartan 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 losartan 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 losartan is not more than 1.0%.

Containers and storage Containers—Tight containers.

Losartan Potassium and Hydrochlorothiazide Tablets

ロサルタンカリウム・ヒドロクロロチアジド錠

Losartan Potassium and Hydrochlorothiazide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$: 461.00) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$: 297.74).

Method of preparation Prepare as directed under Tablets, with Losartan Potassium and Hydrochlorothiazide.

Identification (1) Shake well a portion of powdered Losartan Potassium and Hydrochlorothiazide Tablets, equivalent to 50 mg of Losartan Potassium, with 10 mL of methanol, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of losartan potassium in methanol to make 10 mL. To 5 mL of this solution add methanol to make 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 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 acetic acid (100) (75:25:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the two spots obtained from the sample solution and the spot obtained from the standard solution show the same Rf value.

(2) Shake well a portion of powdered Losartan Potassium and Hydrochlorothiazide Tablets, equivalent to 12.5 mg of Hydrochlorothiazide, with 10 mL of methanol, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of hydrochlorothiazide in methanol to make 10 mL. To 5 mL of this solution add methanol to make 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 ethyl acetate, methanol and acetic acid (100) (75:25:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the two spots obtained from the sample solution and the spot obtained from the standard solution show the same Rf value.

Uniformity of dosage units <6.02>
(1) Losartan potassium—Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets add $V/2$ mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), and stir for

60 minutes to disintegrate the tablet, add sodium dihydrogen phosphate TS (pH 2.5) to make exactly V mL so that each mL contains about 0.5 mg of losartan potassium ($C_{22}H_{22}ClKN_6O$). Pipet 10 mL of this solution, add 45 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) 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 46 mg of Losartan Potassium RS (separately determine the water <2.48> in the same manner as Losartan Potassium), and dissolve in 50 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) to make exactly 100 mL, and use this solution as the losartan potassium standard stock solution. Pipet 12 mL of the losartan potassium standard stock solution, add 44 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) 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 losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$)
$$= M_S \times A_T/A_S \times 3V/250$$

M_S: Amount (mg) of Losartan Potassium RS taken, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 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 35°C.
Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 2.5 with phosphoric acid, and add water to make 1000 mL. To 900 mL of this solution add 600 mL of acetonitrile.
Flow rate: Adjust so that the retention time of losartan is about 5 minutes.
System suitability—
System performance: To 12 mL of the losartan potassium standard stock solution and 4 mL of the hydrochlorothiazide standard stock solution obtained in (2), add 42 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), and add sodium dihydrogen phosphate TS (pH 2.5) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.
(2) Hydrochlorothiazide—Perform the test according to the following method: it meets the requirement of the Content uniformity test.
To 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets add $V/2$ mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), and stir for

60 minutes to disintegrate the tablet, add sodium dihydrogen phosphate TS (pH 2.5) to make exactly V mL so that each mL contains about 0.125 mg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$). Pipet 10 mL of this solution, add 45 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) 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 35 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), and dissolve in 50 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) to make exactly 100 mL, and use this solution as the hydrochlorothiazide standard stock solution. Pipet 4 mL of the hydrochlorothiazide standard stock solution, add 48 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) 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 hydrochlorothiazide in each solution.

Amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)
$$= M_S \times A_T/A_S \times V/250$$

M_S: Amount (mg) of Hydrochlorothiazide RS taken, calculated on the dried basis

Operating conditions—
Proceed as directed in the operating conditions in (1).
System suitability—
System performance: To 12 mL of the losartan potassium standard stock solution obtained in (1) and 4 mL of the hydrochlorothiazide standard stock solution, add 42 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), and add sodium dihydrogen phosphate TS (pH 2.5) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrochlorothiazide is not more than 1.0%.

Dissolution *<6.10>* (1) Losartan potassium—When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Losartan Potassium and Hydrochlorothiazide Tablets is not less than 85%.
Start the test with 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets, 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 2 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 losartan potassium ($C_{22}H_{22}ClKN_6O$), and use this solution as the sample solution. Separately, weigh accurately about 46 mg of Losartan Potassium RS (separately determine the water <2.48> in the same manner as Losartan Potassium), and dissolve in water to make exactly 100 mL, and use this solution

as the losartan potassium standard stock solution. Pipet 12 mL of the losartan potassium standard stock 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 losartan in each solution.

Dissolution rate (%) with respect to the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 108$$

M_S: Amount (mg) of Losartan Potassium RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Uniformity of dosage units (1).
System suitability—
System performance: To 12 mL of the losartan potassium standard stock solution and 8 mL of the hydrochlorothiazide standard stock solution obtained in (2), add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.

(2) Hydrochlorothiazide—When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Losartan Potassium and Hydrochlorothiazide Tablets is not less than 80%.

Start the test with 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets, 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 2 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 13.9 μg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), dissolve in 20 mL of methanol, and add water to make exactly 200 mL, and use this solution as the hydrochlorochiazide standard stock solution. Pipet 8 mL of hydrochlorothiazide standard stock 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 hydrochlorothiazide in each solution.

Dissolution rate (%) with respect to the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$$

M_S: Amount (mg) of Hydrochlorothiazide RS taken, calculated on the dried basis
C: Labeled amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Uniformity of dosage units (1).
System suitability—
System performance: To 12 mL of the losartan potassium standard stock solution obtained in (1) and 8 mL of the hydrochlorothiazide standard stock solution, add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrochlorothiazide is not more than 1.0%.

Assay (1) Losartan potassium—To 10 Losartan Potassium and Hydrochlorothiazide Tablets add 21V/25 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), stir for 60 minutes to disintegrate the tablets, add sodium dihydrogen phosphate TS (pH 2.5) to make exactly V mL so that each mL contains about 2 mg of losartan potassium ($C_{22}H_{22}ClKN_6O$), and treat with ultrasonic waves for 2 minutes. Pipet 10 mL of this solution, add 10 mL of acetonitrile, and add sodium dihydrogen phosphate TS (pH 2.5) to make exactly 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. Separately, weigh accurately about 40 mg of Losartan Potassium RS (separately determine the water <2.48> in the same manner as Losartan Potassium), and dissolve in 30 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) to make exactly 50 mL, and use this solution as the losartan potassium standard stock solution. Pipet 10 mL of the losartan potassium standard stock solution, add 4 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) 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 losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) in 1 tablet
$$= M_S \times A_T/A_S \times V/200$$

M_S: Amount (mg) of Losartan Potassium RS taken, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 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 35°C.
Mobile phase A: Dissolve 1.25 g of potassium dihydrogen phosphate and 1.5 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL. To 930 mL of this solution add 70 mL of acetonitrile.
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 – 12	100 → 92	0 → 8
12 – 28	92 → 38	8 → 62

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

System suitability—

System performance: To 25 mL of the losartan potassium standard stock solution and 10 mL of the hydrochlorothiazide standard stock solution obtained in (2), add sodium dihydrogen phosphate TS (pH 2.5) to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan are eluted in this order, and the number of theoretical plates of the peak of hydrochlorothiazide and the symmetry factor of the peak of losartan are not less than 4000 and not more 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 losartan is not more than 1.0%.

(2) Hydrochlorothiazide—To 10 Losartan Potassium and Hydrochlorothiazide Tablets add 21V/25 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), stir for 60 minutes to disintegrate the tablets, add sodium dihydrogen phosphate TS (pH 2.5) to make exactly V mL so that each mL contains about 0.5 mg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), and treat with ultrasonic waves for 2 minutes. Pipet 10 mL of this solution, add 10 mL of acetonitrile, and add sodium dihydrogen phosphate TS (pH 2.5) to make exactly 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. Separately, weigh accurately about 25 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), and dissolve in a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), to make exactly 50 mL, and use this solution as the hydrochlorothiazide standard stock solution. Pipet 20 mL of the hydrochlorothiazide standard stock solution, add 30 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS (pH 2.5) (3:2), add sodium dihydrogen phosphate TS (pH 2.5) 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 hydrochlorothiazide in each solution.

Amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in 1 tablet
$$= M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of Hydrochlorothiazide RS taken, calculated on the dried basis

Operating conditions—

Proceed as directed in the operating conditions in (1).

System suitability—

System performance: To 25 mL of the losartan potassium standard stock solution obtained in (1) and 10 mL of the hydrochlorothiazide standard stock solution, add sodium dihydrogen phosphate TS (pH 2.5) to make 50 mL. When the procedure is run with 20 μL of this solution under the above

operating conditions, hydrochlorothiazide and losartan are eluted in this order, and the number of theoretical plates of the peak of hydrochlorothiazide and the symmetry factor of the peak of losartan are not less than 4000 and not more 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 hydrochlorothiazide is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Loxoprofen Sodium Hydrate

ロキソプロフェンナトリウム水和物
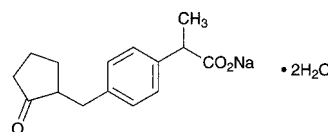
$C_{15}H_{17}NaO_3.2H_2O$: 304.31
Monosodium 2-{4-[(2-oxocyclopentyl)methyl]phenyl}propanoate dihydrate
[*80382-23-6*]

Loxoprofen Sodium Hydrate contains not less than 98.5% of loxoprofen sodium ($C_{15}H_{17}NaO_3$: 268.28), calculated on the anhydrous basis.

Description   Loxoprofen Sodium Hydrate occurs as white to yellowish white, crystals or crystalline powder.

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

A solution of Loxoprofen Sodium Hydrate (1 in 20) does not show optical rotation.

The pH of a solution of 1.0 g of Loxoprofen Sodium Hydrate in 20 mL of freshly boiled and cooled water is between 6.5 and 8.5.

Identification   **(1)**   Determine the absorption spectrum of a solution of Loxoprofen Sodium Hydrate (1 in 55,000) 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 Loxoprofen Sodium Hydrate 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.

(3)   A solution of Loxoprofen Sodium Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Purity   **(1)**   Clarity and color of solution—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of water: the solution is clear and colorless or pale yellow. The color is not darker than that of diluted Matching Fluid A (1 in 2).

(2)   Heavy metals <1.07>—Proceed with 2.0 g of Loxoprofen 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).

(3)   Related substances—Dissolve 1.0 g of Loxoprofen Sodium 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 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 and acetic acid (100) (9:1) 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> 11.0 – 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 60 mg of Loxoprofen Sodium Hydrate, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (3 in 5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Loxoprofen RS, previously dried in a desiccator (in vacuum, 60°C) for 3 hours, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, proceed in the same manner as directed for the preparation of the sample solution, and use so obtained 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 loxoprofen to that of the internal standard.

Amount (mg) of loxoprofen sodium ($C_{15}H_{17}NaO_3$)
= $M_S \times Q_T/Q_S \times 1.089$

M_S: Amount (mg) of Loxoprofen RS taken

Internal standard solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (7 in 50,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 222 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, water, acetic acid (100) and triethylamine (600:400:1:1).
Flow rate: Adjust so that the retention time of loxoprofen 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, loxoprofen 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 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 loxoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Loxoprofen Sodium Tablets

ロキソプロフェンナトリウム錠

Loxoprofen Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of loxoprofen sodium ($C_{15}H_{17}NaO_3$: 268.28).

Method of preparation Prepare as directed under Tablets, with Loxoprofen Sodium Hydrate.

Identification To a quantity of powdered Loxoprofen Sodium Tablets, equivalent to 60 mg of loxoprofen sodium ($C_{15}H_{17}NaO_3$), add 20 mL of methanol, shake vigorously for 10 minutes, and centrifuge. To 1 mL of the supernatant liquid add methanol to make 20 mL. To 2 mL of this solution add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 221 nm and 225 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 Loxoprofen Sodium Tablets add exactly V mL of the internal standard solution so that each mL contains about 3 mg of loxoprofen sodium ($C_{15}H_{17}NaO_3$). After treating with ultrasonic waves for 10 minutes with occasional shaking, centrifuge the solution. To 2 mL of the supernatant liquid add diluted methanol (3 in 5) to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of loxoprofen sodium ($C_{15}H_{17}NaO_3$)
= $M_S \times Q_T/Q_S \times V/10 \times 1.089$

M_S: Amount (mg) of Loxoprofen RS taken

Internal standard solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (3 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 Loxoprofen Sodium Tablets is not less than 85%.
Start the test with 1 tablet of Loxoprofen 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 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 13 μg of loxoprofen sodium ($C_{15}H_{17}NaO_3$). Use this solution as the sample solution. Separately, weigh accurately about 31 mg of Loxoprofen RS, previously dried in vacuum at 60°C for 3 hours, dissolve in 5 mL of ethanol (99.5), and add water to make exactly 250 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test 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 223 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control.

Dissolution rate (%) with respect to the labeled amount of loxoprofen sodium ($C_{15}H_{17}NaO_3$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 36 \times 1.089$

M_S: Amount (mg) of Loxoprofen RS taken
C: Labeled   amount   (mg)   of   loxoprofen   sodium

($C_{15}H_{17}NaO_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Loxoprofen Sodium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 60 mg of loxoprofen sodium ($C_{15}H_{17}NaO_3$), add exactly 20 mL of the internal standard solution, and shake vigorously for 15 minutes. Centrifuge this solution, and to 2 mL of the supernatant liquid add diluted methanol (3 in 5) to make 100 mL. Use this solution as the sample solution. Separately, weigh accurately about 30 mg of Loxoprofen RS, previously dried in vacuum at 60°C for 3 hours, and dissolve in exactly 10 mL of the internal standard solution. To 2 mL of this solution add diluted methanol (3 in 5) 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 loxoprofen to that of the internal standard.

$$\text{Amount (mg) of loxoprofen sodium (}C_{15}H_{17}NaO_3\text{)}$$
$$= M_S \times Q_T/Q_S \times 2 \times 1.089$$

M_S: Amount (mg) of Loxoprofen RS taken

Internal standard solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (3 in 2000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 222 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: A mixture of methanol, water, acetic acid (100) and triethylamine (600:400:1:1).
Flow rate: Adjust so that the retention time of loxoprofen 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, loxoprofen 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 loxoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Lysine Acetate

L-リシン酢酸塩
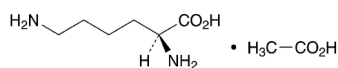
$C_6H_{14}N_2O_2.C_2H_4O_2$: 206.24
(2S)-2,6-Diaminohexanoic acid monoacetate
[57282-49-2]

L-Lysine Acetate, when dried, contains not less than 98.5% and not more than 101.0% of L-lysine acetate ($C_6H_{14}N_2O_2.C_2H_4O_2$).

Description L-Lysine Acetate occurs as white, crystals or crystalline powder. It has a characteristic odor and a slightly acid taste.
It is very soluble in water, freely soluble in formic acid, and practically insoluble in ethanol (99.5).
It is deliquescent.

Identification (1) Determine the infrared absorption spectrum of L-Lysine 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.
(2) A solution of L-Lysine Acetate (1 in 20) responds to the Qualitative Tests <1.09> (2) for acetate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +8.5 – +10.0° (after drying, 2.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the pH of the solution is between 6.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the solution is colorless and clear.
(2) Chloride <1.03>—Perform the test with 0.5 g of L-Lysine Acetate. 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-Lysine Acetate. 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-Lysine Acetate. 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 L-Lysine Acetate according to Method 4, 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-Lysine Acetate 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-Lysine Acetate, 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 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately 2.5 mmol amounts 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 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 this solution, add 0.02 mol/L hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid 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. Based on the peak heights of the amino acids obtained from the sample solution and standard solution, determine the mass of the amino acids other than lysine contained in 1 mL of the sample solution, and calculate the mass percent: the amount of each amino acids other than lysine is not more than 0.1%.
Operating conditions—
Detector: A visible spectrophotometer (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 copolymer (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.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and to each phase add 0.1 mL of capric acid.

	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	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount
Total volume	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Changing mobile phases: Proceed with 20 μL of the standard solution under the above operating 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. Switchover the mobile phases A, B, C, D and E in sequence so that the resolution between the peaks of isoleucine and leucine is not less than 1.2.

Reaction reagents: Dissolve 204 g of lithium acetate dihydrate in water, and add 123 mL of acetic acid (100), 401 mL of 1-methoxy-2-propanol, and water to make 1000 mL, gas with nitrogen for 10 minutes, and use this solution as the solution (I). Separately, to 979 mL of 1-methoxy-2-propanol add 39 g of ninhydrin, gas with nitrogen for 5 minutes, add 81 mg of sodium borohydride, gas the solution with nitrogen for 30 minutes, and use this solution as solution (II). To 1 volume of the solution (I) add 1 volume of the solution (II). Prepare before use.

Mobile phase flow rate: 0.20 mL per minute.

Reaction reagent flow rate: 0.24 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and 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 is not more than 1.0%.

Loss on drying <2.41>   Not more than 0.3% (1 g, 80°C, 3 hours).

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

Assay   Weigh accurately about 0.1 g of L-Lysine Acetate, 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.
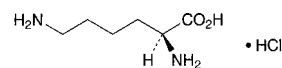
Each mL of 0.1 mol/L perchloric acid VS
= 10.31 mg of $C_6H_{14}N_2O_2.C_2H_4O_2$

Containers and storage   Containers—Tight containers.

L-Lysine Hydrochloride

Lysine Hydrochloride

L-リシン塩酸塩

$C_6H_{14}N_2O_2.HCl$: 182.65
(2S)-2,6-Diaminohexanoic acid monohydrochloride
[657-27-2]

L-Lysine Hydrochloride, when dried, contains not less than 98.5% of L-lysine hydrochloride ($C_6H_{14}N_2O_2.HCl$).

Description   L-Lysine Hydrochloride occurs as a white powder. It has a slight, characteristic taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

It shows crystal polymorphism.

Identification   (1)   Determine the infrared absorption spectrum of L-Lysine 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. If any difference appears between the spectra, dissolve L-Lysine Hydrochloride in water, evaporate the water to dryness at 60°C, and repeat the test with the residue.

(2)   A solution of L-Lysine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +19.0 – +21.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54>   Dissolve 1.0 g of L-Lysine Hydrochloride in 10 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 L-Lysine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2)   Sulfate <1.14>—Perform the test with 0.6 g of L-Lysine Hydrochloride. 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-Lysine Hydrochloride. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4)   Heavy metals <1.07>—Proceed with 2.0 g of L-Lysine 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).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of L-Lysine Hydrochloride 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-propanol and ammonia water (28) (67:33) to a distance of about 10 cm, and dry the plate at 100°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 1.0% (1 g, 105°C, 3 hours).

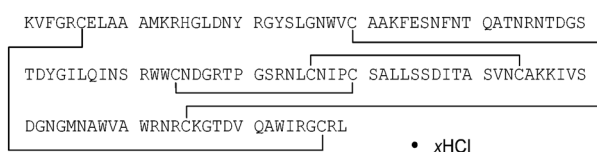**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Lysine Hydrochloride, previously dried, dissolve in 2 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 perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 9.132 \text{ mg of } C_6H_{14}N_2O_2.HCl$$

Containers and storage   Containers—Tight containers.

Lysozyme Hydrochloride

リゾチーム塩酸塩

```
KVFGRCELAA AMKRHGLDNY RGYSLGNWVC AAKFESNFNT QATNRNTDGS

TDYGILQINS RWWCNDGRTP GSRNLCNIPC SALLSSDITA SVNCAKKIVS

DGNGMNAWVA WRNRCKGTDV QAWIRGCRL        • xHCl
```

$C_{616}H_{963}N_{193}O_{182}S_{10}.x$HCl
[*12650-88-3*, egg white lysozyme]

Lysozyme Hydrochloride is a hydrochloride of a basic polypeptide obtained from albumen of hen's egg, and has an activity to hydrolyze mucopolysaccharides.

It contains not less than 0.9 mg (potency) of lysozyme per mg, calculated on the dried basis.

Description Lysozyme Hydrochloride occurs as white, crystals, or crystalline or amorphous powder.

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

It is hygroscopic.

The pH of a solution of 3 g of Lysozyme Hydrochloride in 200 mL of water is between 3.0 and 5.0.

Identification **(1)** To 5 mL of a solution of Lysozyme Hydrochloride in acetate buffer solution (pH 5.4) (1 in 500) add 1 mL of ninhydrin TS, and heat for 10 minutes: a blue-pur-

ple color develops.

(2) Determine the absorption spectrum of a solution of Lysozyme Hydrochloride in acetate buffer solution (pH 5.4) (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.

Purity **(1)** Clarity of solution—To 5 mL of a solution of Lysozyme Hydrochloride (3 in 200) add, if necessary, dilute hydrochloric acid to adjust the pH to 3: the solution is clear.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Lysozyme 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 8.0% (0.1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 2.0% (0.5 g).

Nitrogen Perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is between 16.8% and 18.6%, calculated on the dried basis.

Assay Weigh accurately an amount of Lysozyme Hydrochloride, equivalent to about 25 mg (potency), dissolve in phosphate buffer solution (pH 6.2) to make exactly 100 mL. Pipet 2 mL of this solution, add phosphate buffer solution (pH 6.2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lysozyme RS (separately determine its loss on drying <*2.41*> under the same condition as Lysozyme Hydrochloride), equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution (pH 6.2) to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, add phosphate buffer solution (pH 6.2) to them to make exactly 50 mL, and use these solutions as the standard solution (1) and the solution (2), respectively. Keep the sample solution and the standard solutions in an ice-bath. Pipet 4 mL of substrate solution for lysozyme hydrochloride, previously warmed in a water bath of 35°C for about 5 minutes, add exactly 100 μL of the sample solution, previously warmed in a water bath of 35°C for about 3 minutes, and allow to stand at 35°C for exactly 10 minutes, then add exactly 0.5 mL of 1 mol/L hydrochloric acid TS, and immediately shake. Determine the absorbance under Ultraviolet-visible Spectrophotometry <*2.24*>, A_T, of this solution at 640 nm, using water as the blank. Determine the absorbances, A_{S1} and A_{S2}, of the solutions obtained with the standard solution (1) and the standard solution (2) in the same manner as the sample solution.

$$\text{Amount [mg (potency)] of lysozyme per mg,}$$
$$\text{calculated on the dried basis}$$
$$= M_S/2M_T \times \{(A_{S1} - A_T)/(A_{S1} - A_{S2}) + 1\}$$

M_S: Amount (mg) of Lysozyme RS taken, calculated on the dried basis.

M_T: Amount (mg) of the sample taken, calculated on the dried basis.

Containers and storage   Containers—Tight containers.

Macrogol 400

Polyethylene Glycol 400

マクロゴール **400**

Macrogol 400 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2$ $(CH_2OCH_2)_nCH_2OH$, in which the value of n ranges from 7 to 9.

Description   Macrogol 400 occurs as a clear, colorless and viscous liquid. It has no odor or a slight, characteristic odor.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is soluble in diethyl ether.

It is slightly hygroscopic.

Congealing point: 4 – 8°C

Specific gravity d_{20}^{20}: 1.110 – 1.140

Identification   Dissolve 50 mg of Macrogol 400 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n-hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54>   Dissolve 1.0 g of Macrogol 400 in 20 mL of water: the pH of this solution is between 4.0 and 7.0.

Purity   **(1)**   Acidity—Dissolve 5.0 g of Macrogol 400 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2)   Ethylene glycol and diethylene glycol—Dissolve 4.0 g of Macrogol 400 in water to make exactly 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg each of ethylene glycol and diethylene glycol, dissolve in water 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 Gas Chromatography <2.02> according to the following conditions. Determine the peak heights, H_{Ta} and H_{Sa}, of ethylene glycol of each solution, and the peak heights, H_{Tb} and H_{Sb}, of diethylene glycol, and calculate the amount of ethylene glycol and diethylene glycol: the sum of the contents of ethylene glycol and diethylene glycol is not more than 0.25%.

$$\text{Amount (mg) of ethylene glycol} = M_{Sa} \times H_{Ta}/H_{Sa} \times 1/10$$

$$\text{Amount (mg) of diethylene glycol} = M_{Sb} \times H_{Tb}/H_{Sb} \times 1/10$$

M_{Sa}: Amount (mg) of ethylene glycol taken

M_{Sb}: Amount (mg) of diethylene glycol taken

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A colum about 3 mm in inside diameter and about 1.5 m in length, packed with siliceous earth for gas chromatography, 150 to 180 μm in particle diameter, coated with D-sorbitol for gas chromatography at the ratio of 12%.

Column temperature: A constant temperature of about 165°C.

Carrier gas: Nitrogen or helium.

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

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column clearly dividing peaks of ethylene glycol and diethylene glycol in this order.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diethylene glycol obtained from 2 μL of the standard solution composes about 80% of the full scale.

Average molecular mass   Add 42 g of phthalic anhydride to 300 mL of freshly distilled pyridine, exactly measured, in a 1-L light-resistant glass-stoppered bottle. Shake the bottle vigorously to dissolved the solid, and allow to stand for 16 hours or more. Pipet 25 mL of this solution into an about 200-mL glass-stoppered pressure bottle. Add about 1.5 g of Macrogol 400, accurately weighed, stopper the bottle, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of Macrogol 400 taken

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination

b: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 380 and 420.

Water <2.48>   Not more than 1.0% (2 g, volumetric titration, direct titration).

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

Containers and storage   Containers—Tight containers.

Macrogol 1500

Polyethylene Glycol 1500

マクロゴール **1500**

Macrogol 1500 is a mixture containing equal amounts of lower and higher polymers of ethylene oxide and water, represented by the formula $HOCH_2$ $(CH_2OCH_2)_nCH_2OH$, in which the value of n is 5 or 6 for the lower polymers and from 28 to 36 for the higher.

Description   Macrogol 1500 occurs as a white, smooth petrolatum-like solid. It is odorless or has a faint, characteristic odor.

It is very soluble in water, in pyridine and in diphenyl ether, freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Congealing point: 37 – 41°C

Identification   Dissolve 50 mg of Macrogol 1500 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n-hydrate (1 in 10): a yellow-green precipitate is formed.

pH *<2.54>* Dissolve 1.0 g of Macrogol 1500 in 20 mL of water: the pH of the solution is between 4.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 5.0 g of Macrogol 1500 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 1500 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(3) Ethylene glycol and diethylene glycol—Place 50.0 g of Macrogol 1500 in a distilling flask, add 75 mL of diphenyl ether, warm to dissolve if necessary, distil slowly under a reduced pressure of 0.13 to 0.27 kPa and take 25 mL of the distillate in a 100-mL container with 1-mL graduation. To the distillate add exactly 20 mL of water, shake vigorously, cool in ice water, congeal the diphenyl ether, and filtrate into a 25-mL volumetric flask. Wash the residue with 5.0 mL of ice-cold water, combine the washings with the filtrate, warm to room temperature, and add water to make 25 mL. Transfer this solution to a glass-stoppered flask, shake with 25.0 mL of freshly distilled acetonitrile, and use this solution as the sample solution. Separately, to 62.5 mg of diethylene glycol add a mixture of water and freshly distilled acetonitrile (1:1) to make exactly 25 mL, and use this solution as the standard solution. Take exactly 10 mL each of the sample solution and the standard solution, and add to each exactly 15 mL of cerium (IV) diammonium nitrate TS. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>* within 2 to 5 minutes: the absorbance of the solution obtained from the sample solution at the wavelength of maximum absorption at about 450 nm is not larger than the absorbance of the solution obtained from the standard solution.

Water *<2.48>* Not more than 1.0% (2 g, volumetric titration, direct titration).

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

Containers and storage Containers—Tight containers.

Macrogol 4000

Polyethylene Glycol 4000

マクロゴール **4000**

Macrogol 4000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of n ranges from 59 to 84.

Description Macrogol 4000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in methanol and in pyridine, and practically insoluble in ethanol (99.5) and in diethyl ether.

Congealing point: 53 – 57°C

Identification Dissolve 50 mg of Macrogol 4000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH *<2.54>* Dissolve 1.0 g of Macrogol 4000 in 20 mL of water: the pH of this solution is between 4.0 and 7.5.

Purity (1) Clarity and color of solution—A solution of 5.0 g of Macrogol 4000 in 50 mL of water is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 4000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthelein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 12.5 g of Macrogol 4000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1-L light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate *<2.50>* with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of Macrogol 4000 taken
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 2600 and 3800.

Water *<2.48>* Not more than 1.0% (2 g, volumetric titration, direct titration).

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

Containers and storage Containers—Well-closed containers.

Macrogol 6000

Polyethylene Glycol 6000

マクロゴール **6000**

Macrogol 6000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of n ranges from 165 to 210.

Description Macrogol 6000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in pyridine, and practically insoluble in methanol, in ethanol (95), in ethanol (99.5) and in diethyl ether.

Congealing point: 56 – 61°C

Identification Dissolve 50 mg of Macrogol 6000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54>   Dissolve 1.0 g of Macrogol 6000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity   (1)   Clarity and color of solution—Dissolve 5.0 g of Macrogol 6000 in 50 mL of water: the solution is clear and colorless.

(2)   Acidity—Dissolve 5.0 g of Macrogol 6000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass   Weigh accurately about 12.5 g of Macrogol 6000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1-L light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination in the same manner.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of Macrogol 6000 taken
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample

Average molecular mass is between 7300 and 9300.

Water <2.48>   Not more than 1.0% (2 g, volumetric titration, direct titration).

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

Containers and storage   Containers—Well-closed containers.

Macrogol 20000

Polyethylene Glycol 20000

マクロゴール **20000**

Macrogol 20000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2$ $(CH_2OCH_2)_nCH_2OH$, in which the value of n lies between 340 and 570.

Description   Macrogol 20000 occurs as white, paraffin-like flakes or powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water and in pyridine, and practically insoluble in methanol, in ethanol (95), in petroleum benzine and in macrogol 400.

Congealing point: 56 – 64°C

Identification   Dissolve 50 mg of Macrogol 20000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS,

shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54>   Dissolve 1.0 g of Macrogol 20000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity   (1)   Clarity and color of solution—Dissolve 5.0 g of Macrogol 20000 in 50 mL of water: the solution is clear and colorless.

(2)   Acidity—Dissolve 5.0 g of Macrogol 20000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass   Weigh accurately about 15 g of Macrogol 20000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1-L light-resistant glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the same depth as the mixture in the bottle. Maintain the temperature of the bath at 98 ± 2°C for 60 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (M \times 4000)/(a - b)$$

M: Amount (g) of Macrogol 20000 taken
a: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination
b: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample

Average molecular mass is between 15000 and 25000.

Water <2.48>   Not more than 1.0% (2 g, volumetric titration, direct titration).

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

Containers and storage   Containers—Well-closed containers.

Macrogol Ointment

Polyethylene Glycol Ointment

マクロゴール軟膏

Method of preparation

Macrogol 4000	500 g
Macrogol 400	500 g
	To make 1000 g

Melt Macrogol 4000 and Macrogol 400 by warming on a water bath at 65°C, and mix well until it congeals. Less than 100 g of Macrogol 4000 or Macrogol 400 may be replaced by an equal amount of Macrogol 400 or Macrogol 4000 to prepare 1000 g of a proper soft ointment.

Description Macrogol Ointment is white in color. It has a faint, characteristic odor.

Identification Dissolve 50 mg of Macrogol Ointment in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, filter if necessary, and add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10) to the filtrate: a yellow-green precipitate is formed.

Containers and storage Containers—Tight containers.

Magnesium Carbonate

炭酸マグネシウム

Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate.

Magnesium Carbonate contains not less than 40.0% and not more then 44.0% of magnesium oxide (MgO: 40.30).

"Heavy magnesium carbonate" may be used as commonly used name for Magnesium Carbonate which shows the height of the precipitate below the 12.0-mL graduation line in the Precipitation test.

Description Magnesium Carbonate occurs as white, friable masses or powder. It is odorless.

It is practically insoluble in water, in ethanol (95), in 1-propanol and in diethyl ether.

It dissolves in dilute hydrochloric acid with effervescence.

Its saturated solution is alkaline.

Identification (1) Dissolve 1 g of Magnesium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, neutralize with sodium hydroxide TS, and filter, if necessary: the solution responds to the Qualitative Tests <*1.09*> for magnesium salt.

(2) Magnesium Carbonate responds to the Qualitative Tests <*1.09*> (1) for carbonate.

Purity (1) Soluble salts—To 2.0 g of Magnesium Carbonate add 40 mL of 1-propanol and 40 mL of water, heat to boil with constant stirring, cool, and filter. Wash the residue with water, combine the washings with the filtrate, and add water to make exactly 100 mL. Evaporate 50 mL of the solution on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue does not exceed 10.0 mg.

(2) Heavy metals <*1.07*>—Moisten 1.0 g of Magnesium Carbonate with 4 mL of water, dissolve by addition of 10 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid, 1 drop of ammonia TS, filter, if necessary, wash the filter paper with water, combine the washings with the filtrate, and add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 30 ppm).

(3) Iron <*1.10*>—Prepare the test solution with 0.10 g of Magnesium Carbonate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 200 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Magnesium Carbonate, previously moistened with 1.5 mL of water, add 3.5 mL of dilute hydrochloric acid, and perform the test (not more than 5 ppm).

(5) Calcium oxide—Weigh accurately about 0.6 g of Magnesium Carbonate, and dissolve in 35 mL of water and 6 mL of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2″-nitrilotriethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <*2.50*> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes form red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

> Each mL of 0.01 mol/L disodium dihydrogen
> ethylenediamine tetraacetate VS
> = 0.5608 mg of CaO

The content of calcium oxide (CaO: 56.08) is not more than 0.6%.

(6) Acid-insoluble substances—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid dropwise while stirring, boil for 5 minutes, and cool. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 2.5 mg.

Precipitation test Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 (150 μm) sieve to a glass-stoppered measuring cylinder with a 50-mL graduation line at 150 mm from the bottom, and add water to make 50 mL. Shake vigorously for exactly 1 minute, allow to stand for 15 minutes, and measure the height of the precipitate (in graduation in ml).

Assay Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7) and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

> Each mL of 0.05 mol/L disodium dihydrogen
> ethylenediamine tetraacetate VS
> = 2.015 mg of MgO

> Each mg of calcium oxide (CaO)
> = 0.36 mL of 0.05 mol/L disodium dihydrogen
> ethylenediamine tetraacetate VS

Containers and storage Containers—Well-closed containers.

Magnesium Oxide

酸化マグネシウム

MgO: 40.30

Magnesium Oxide, when ignited, contains not less than 96.0% of magnesium oxide (MgO).

When 5 g of Magnesium Oxide has a volume not more than 30 mL, it may be labeled heavy magnesium oxide.

Description Magnesium Oxide occurs as a white, powder or granules. It is odorless.

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

It dissolves in dilute hydrochloric acid.

It absorbs moisture and carbon dioxide in air.

Identification A solution of Magnesium Oxide in dilute hydrochloric acid (1 in 50) responds to the Qualitative Tests <*1.09*> for magnesium salt.

Purity **(1)** Alkali and soluble salts—Transfer 2.0 g of Magnesium Oxide to a beaker, add 100 mL of water, cover the beaker with a watch-glass, heat on a water bath for 5 minutes, and filter immediately. After cooling, to 50 mL of the filtrate add 2 drops of methyl red TS and 2.0 mL of 0.05 mol/L sulfuric acid VS: a red color develops. Evaporate 25 mL of the remaining filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 10 mg.

(2) Carbonate—Boil 0.10 g of Magnesium Oxide with 5 mL of water, cool, and add 5 mL of acetic acid (31): almost no effervescence occurs.

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Magnesium Oxide in 20 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 1 drop of phenolphthalein TS, neutralize with ammonia TS, add 2 mL of dilute acetic acid, and filter, if necessary. Wash the filter paper with water, add water to the combined washing and the filtrate to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 20 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, neutralize with ammonia TS, and add 2 mL of dilute acetic acid, 4.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(4) Iron <*1.10*>—Prepare the test solution with 40 mg of Magnesium Oxide according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(5) Calcium oxide—Weigh accurately about 0.25 g of Magnesium Oxide, previously ignited, dissolve in 6 mL of dilute hydrochloric acid by heating. Cool, add 300 mL of water and 3 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2″-nitrilotriethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and 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 (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.
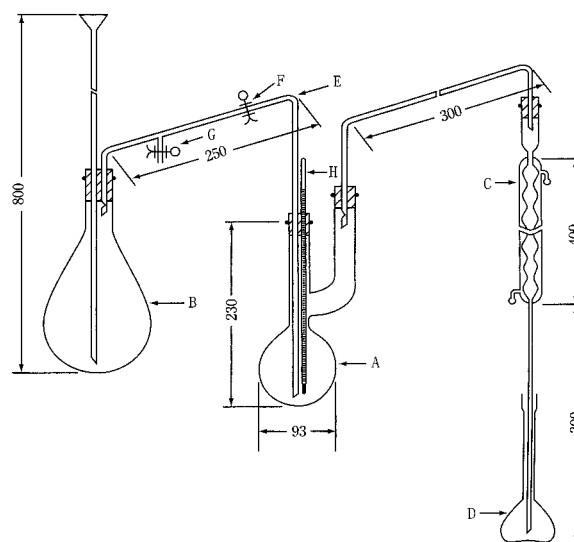
The figures are in mm.

A: Distilling flask of about 300-mL capacity.
B: Steam generator of about 1000-mL capacity, containing a few boiling tips to prevent bumping
C: Condenser
D: Receiver: 200-mL volumetric flask
E: Steam-introducing tube having an internal diameter of about 8 mm
F, G: Rubber tube with a clamp
H: Thermometer

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.5608 mg of CaO

The mass of calcium oxide (CaO: 56.08) is not more than 1.5%.

(6) Arsenic <*1.11*>—Dissolve 0.20 g of Magnesium Oxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 10 ppm).

(7) Acid-insoluble substances—Mix 2.0 g of Magnesium Oxide with 75 mL of water, add 12 mL of hydrochloric acid dropwise, while shaking, and boil for 5 minutes. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the ignited residue does not more than 2.0 mg.

(8) Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(ii) Procedure: Transfer 5.0 g of Magnesium Oxide to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130°C, then open the rubber tube F, close the rubber tube G, boil water in the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A, and maintain the temperature of the solution in A between 135°C and 145°C. Adjust the distilling rate to about 10 mL per minute. Collect about

170 mL of the distillate, then stop the distillation, wash C with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL, and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for fluoride under Oxygen Flask Combustion Method <*1.06*>. No corrective solution is used in this procedure. The content of fluoride (F) is not more than 0.08%.

> Amount (mg) of fluoride (F: 19.00) in the test solution
> = amount (mg) of fluoride in 5 mL of
>   the standard solution
> $\times A_T/A_S \times 200/V$

Loss on ignition <*2.43*>   Not more than 10% (0.25 g, 900°C, constant mass).

Assay   Ignite Magnesium Oxide to constant mass at 900°C, weigh accurately about 0.2 g of the residue, dissolve in 10 mL of water and 4.0 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7) and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed, deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

> Each mL of 0.05 mol/L disodium dihydrogen
>   ethylenediamine tetraacetate VS
>   = 2.015 mg of MgO

> Each mg of calcium oxide (CaO)
>   = 0.36 mL of 0.05 mol/L disodium dihydrogen
>   ethylenediamine tetraacetate VS

Containers and storage   Containers—Tight containers.

Magnesium Silicate

ケイ酸マグネシウム

Magnesium Silicate contains not less than 45.0% of silicon dioxide (SiO$_2$: 60.08) and not less than 20.0% of magnesium oxide (MgO: 40.30), and the ratio of percentage (%) of magnesium oxide to silicon dioxide is not less than 2.2 and not more than 2.5.

Description   Magnesium Silicate occurs as a white, fine powder. It is odorless and tasteless.

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

Identification   (1)   Mix 0.5 g of Magnesium Silicate with 10 mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for magnesium salt.

(2)   Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Magnesium Silicate, and fuse again: an infusible matter appears in the bead, which changes to an opaque bead with a web-like structure upon cooling.

Purity   (1)   Soluble salts—Add 150 mL of water to 10.0 g of Magnesium Silicate, heat on a water bath for 60 minutes with occasional shaking, then cool, dilute with water to 150 mL, and centrifuge. Dilute 75 mL of the resultant transparent liquid with water to 100 mL, and use this solution as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 0.02 g.

(2)   Alkalinity—To 20 mL of the sample solution obtained in (1) add 2 drops of phenolphthalein TS and 1.0 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

(3)   Chloride <*1.03*>—Take 10 mL of the sample solution obtained in (1), add 6 mL of dilute nitric acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(4)   Sulfate <*1.14*>—To the residue obtained in (1) add about 3 mL of dilute hydrochloric acid, and heat on a water bath for 10 minutes. Add 30 mL of water, filter, wash the residue on the filter with water, combine the washings with the filtrate, and dilute to 50 mL with water. To 4 mL of the 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 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(5)   Heavy metals <*1.07*>—To 1.0 g of Magnesium Silicate add 20 mL of water and 3 mL of hydrochloric acid, and boil for 2 minutes. Filter, and wash the residue on the filter with two 5-mL portions of water. Evaporate the combined filtrate and washings on a water bath to dryness, add 2 mL of dilute acetic acid to the residue, warm until solution is complete, filter, if necessary, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

(6)   Arsenic <*1.11*>—To 0.40 g of Magnesium Silicate add 5 mL of dilute hydrochloric acid, heat gently to boiling while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 5 ppm).

Loss on ignition <*2.43*>   Not more than 34% (0.5 g, 850°C, 3 hours).

Acid-consuming capacity <*6.04*>   Place about 0.2 g of Magnesium Silicate, accurately weighed, in a glass-stoppered flask, add exactly 30 mL of 0.1 mol/L hydrochloric acid VS and 20 mL of water, shake at 37 ± 2°C for 1 hour, and cool. Pipet 25 mL of the supernatant liquid, and titrate <*2.50*> the excess hydrochloric acid, while stirring well, with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5.

1 g of Magnesium Silicate, calculated on the anhydrous basis by making allowance for the observed loss on ignition determined as directed in the preceding Loss on ignition, consumes not less than 140 mL and not more than 160 mL of 0.1 mol/L hydrochloric acid VS.

Assay   (1)   Silicon dioxide—Weigh accurately about 0.7 g of Magnesium Silicate, add 10 mL of 0.5 mol/L sulfuric acid TS, evaporate on a water bath to dryness, add 25 mL of water to the residue, and heat on a water bath for 15 minutes with occasional stirring. Filter the supernatant liquid through filter paper for assay, add 25 mL of hot water to the residue, stir, and decant the supernatant liquid on the filter

paper to filter. Wash the residue in the same manner with two 25-mL portions of hot water, transfer the residue onto the filter paper, and wash with hot water until the last washing does not respond to the Qualitative Tests <1.09> (1) for sulfate. Place the residue and the filter paper in a platinum crucible, incinerate with strong heating, and ignite between 775°C and 825°C for 30 minutes, then cool, and weigh the residue as a (g). Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh the residue as b (g).

$$\text{Content (\%) of silicon dioxide (SiO}_2)$$
$$= (a - b)/M \times 100$$

M: Mass (g) of the Magnesium Silicate taken

(2)   Magnesium oxide—Weigh accurately about 0.3 g of Magnesium Silicate, transfer to a 50-mL conical flask, add 10 mL of 0.5 mol/L sulfuric acid VS, and heat on a water bath for 15 minutes. Cool, transfer to a 100-mL volumetric flask, wash the conical flask with water, add the washings to the volumetric flask, dilute with water to 100 mL, and filter. Pipet 50 mL of the filtrate, shake with 50 mL of water and 5 mL of diluted 2,2′,2″-nitrilotriethanol (1 in 2), add 2.0 mL of ammonia TS and 10 mL of ammonia-ammonium chloride buffer solution (pH 10.7) and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen
ethylenediamine tetraacetate VS
= 2.015 mg of MgO

(3)   Ratio of percentage (%) of magnesium oxide (MgO) to silicon dioxide (SiO$_2$)—Calculate the quotient from the percentages obtained in (1) and (2).

Containers and storage   Containers—Well-closed containers.

Magnesium Stearate

ステアリン酸マグネシウム

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 (◆   ◆).

Magnesium Stearate is a compound of magnesium with a mixture of solid fatty acids, and consists chiefly of variable proportions of magnesium stearate and magnesium palmitate obtained from sources of vegetable or animal origin.

It contains not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31), calculated on the dried basis.

◆**Description**   Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

It is practically insoluble in water and in ethanol (99.5).◆

Identification   Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cool-

ing, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make 50 mL, and use this solution as the sample solution. To 1 mL of the sample solution add 1 mL of ammonia TS: A white precipitate is formed that dissolves on addition of 1 mL of ammonium chloride TS. By further addition of 1 mL of a solution of disodium hydrogen phosphate dodecahydrate (4 in 25) a white crystalline precipitate is formed.

Purity   (1)   Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on a water bath for 1 minute while shaking, cool, and filter. Add 0.05 mL of bromothymol blue TS to 10 mL of the filtrate: not more than 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS is required to change the color of the solution.

(2)   Chloride <1.03>—Perform the test with 10.0 mL of the sample solution obtained in Identification. Prepare the control solution with 1.4 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.1%).

(3)   Sulfate <1.14>—Perform the test with 6.0 mL of the sample solution obtained in Identification. Prepare the control solution with 3.0 mL of 0.02 mol/L sulfuric acid VS (not more than 1.0%).

◆(4)   Heavy metals <1.07>—Heat 1.0 g of Magnesium Stearate weakly first, then incinerate at about 500 ± 25°C. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, add 20 mL of water and 2 mL of dilute acetic acid to the residue, and heat for 2 minutes. After cooling, filter this solution through a filter paper, wash the filter paper with 15 mL of water, and combine the washing with the filtrate. To the filtrate add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).◆

Loss on drying <2.41>   Not more than 6.0% (2 g, 105°C, constant mass).

◆**Microbial limit** <4.05>   The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 5×10^2 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.◆

Relative content of stearic acid and palmitic acid   Transfer 0.10 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for 10 minutes to dissolve the solids. Add 4 mL of heptane through the condenser, and reflux for 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Pass the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, into another flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, and use this solution as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas chromatography <2.02> according to the following conditions, and determine the area, A, of the methyl stearate peak and the sum of the areas, B, of all of the fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

Content (%) of stearic acid = $A/B \times 100$

Similarly, calculate the percentage of palmitic acid in the portion of Magnesium Stearate taken. The methyl stearate peak, and the sum of the stearate and palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5-μm layer of polyethylene glycol 15000-diepoxide for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain 240°C for 5 minutes.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 260°C.

Carrier gas: Helium.

Flow rate: 2.4 mL per minute.

Split ratio: Splitless.

♦Time span of measurement: For 41 minutes after the solvent peak.♦

System suitability—

♦Test for required detectability:♦ Place about 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. ♦To exactly 1 mL of the solution for system suitability test add heptane to make exactly 10 mL. To exactly 1 mL of this solution add heptane to make exactly 10 mL. Further, to exactly 1 mL of this solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained from 1 μL of this solution is equivalent to 0.05 to 0.15% of that obtained from 1 μL of the solution for system suitability test.♦

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, the relative retention time of methyl palmitate to methyl stearate is about 0.9, and the resolution between these peaks is not less than 5.0.

System repeatability: When the test is repeated 6 times with the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate are not more than 3.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

Assay Transfer about 0.5 g of Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of ethanol (99.5) and 1-butanol (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution (pH 10), 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45 – 50°C to make the solution clear, and after cooling, titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to violet in color. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
    = 2.431 mg of Mg

♦**Containers and storage** Containers—Tight containers.♦

Magnesium Sulfate Hydrate

硫酸マグネシウム水和物

MgSO$_4$.7H$_2$O: 246.47

Magnesium Sulfate Hydrate, when ignited, contains not less than 99.0% of magnesium sulfate (MgSO$_4$: 120.37).

Description Magnesium Sulfate Hydrate occurs as colorless or white crystals. It has a cooling, saline, bitter taste.

It is very soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification A solution of Magnesium Sulfate Hydrate (1 in 40) responds to the Qualitative Tests <1.09> for magnesium salt and for sulfate.

pH <2.54> Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the pH of this solution is between 5.0 and 8.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 1.0 g of Magnesium Sulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Magnesium Sulfate 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) Zinc—Dissolve 2.0 g of Magnesium Sulfate Hydrate in 20 mL of water, and add 1 mL of acetic acid (31) and 5 drops of potassium hexacyanoferrate (II) TS: no turbidity is produced.

(5) Calcium—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 5.0 mL of dilute hydrochloric acid, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 1.0 g of Magnesium Sulfate Hydrate in 2.0 mL of Standard Calcium Solution and 5.0 mL of dilute hydrochloric acid, 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 Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the absorbances, A_T and A_S, of both solutions: A_T is smaller than $A_S - A_T$ (not more than 0.02%).

Gas: Combustible gas—Acetylene or hydrogen.
    Supporting gas—Air.

Lamp: Calcium hollow-cathod lamp.

Wavelength: 422.7 nm.

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on ignition <2.43> 45.0 – 52.0% (1 g, after drying at 105°C for 2 hours, ignite at 450°C for 3 hours).

Assay Weigh accurately about 0.6 g of Magnesium Sulfate

Hydrate, previously ignited at 450°C for 3 hours after drying at 105°C for 2 hours, and dissolve in 2 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen
ethylenediamine tetraacetate VS
= 6.018 mg of $MgSO_4$

Containers and storage   Containers—Well-closed containers.

Magnesium Sulfate Injection

硫酸マグネシウム注射液

Magnesium Sulfate Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of magnesium sulfate hydrate ($MgSO_4.7H_2O$: 246.47).

Method of preparation   Prepare as directed under Injections, with Magnesium Sulfate Hydrate.

Description   Magnesium Sulfate Injection is a clear, colorless liquid.

Identification   Measure a volume of Magnesium Sulfate Injection, equivalent to 0.5 g of Magnesium Sulfate Hydrate, and add water to make 20 mL: the solution responds to the Qualitative Tests <*1.09*> for magnesium salt and for sulfate.

pH <*2.54*>   5.5 – 7.0   When the labeled concentration exceeds 5%, prepare a solution of 5% with water, and perform the test.

Bacterial endotoxins <*4.01*>   Less than 0.09 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 Magnesium Sulfate Injection, equivalent to about 0.3 g of magnesium sulfate hydrate ($MgSO_4.7H_2O$), and add water to make 75 mL. Then add 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and proceed as directed in the Assay under Magnesium Sulfate Hydrate.

Each mL of 0.05 mol/L disodium dihydrogen
ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4.7H_2O$

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

Magnesium Sulfate Mixture

硫酸マグネシウム水

Magnesium Sulfate Mixture contains not less than 13.5 w/v% and not more than 16.5 w/v% of magnesium sulfate hydrate ($MgSO_4.7H_2O$: 246.47).

Method of preparation

Magnesium Sulfate Hydrate	150 g
Bitter Tincture	20 mL
Dilute Hydrochloric Acid	5 mL
Purified Water or Purified	
Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare before use, with the above ingredients.

Description   Magnesium Sulfate Mixture is a light yellowish clear liquid. It has a bitter and acid taste.

Identification   (1)   Magnesium Sulfate Mixture responds to the Qualitative Tests <*1.09*> for magnesium salt.
(2)   Magnesium Sulfate Mixture responds to the Qualitative Tests <*1.09*> (2) for chloride.

Assay   Pipet 10 mL of Magnesium Sulfate Mixture, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen
ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4.7H_2O$

Containers and storage   Containers—Tight containers.

Maltose Hydrate

マルトース水和物
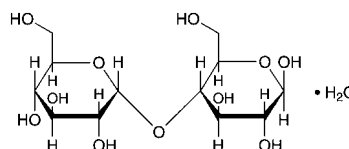
$C_{12}H_{22}O_{11}.H_2O$: 360.31
α-D-Glucopyranosyl-(1→4)-β-D-glucopyranose
monohydrate
[*6363-53-7*]

Maltose Hydrate, when dried, contains not less than 98.0% of maltose hydrate ($C_{12}H_{22}O_{11}.H_2O$).

Description   Maltose Hydrate occurs as white, crystals or crystalline powder. It has a sweet taste.
It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification   (1)   Dissolve 0.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of ammonia TS, and heat for 5 minutes on a water bath: an orange color develops.
(2)   Add 2 to 3 drops of a solution of Maltose Hydrate (1 in 50) to 5 mL of boiling Fehling TS: a red precipitate is

formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: +126 – +131° Weigh accurately about 10 g of Maltose Hydrate, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

pH <2.54> The pH of a solution of 1.0 g of Maltose Hydrate in 10 mL of water is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Put 10 g of Maltose Hydrate in 30 mL of water in a Nessler tube, warm at 60°C in a water bath to dissolve, and after cooling, add water to make 50 mL: the solution is clear, and has no more color than the following control solution.

Control solution: Add water 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 to make 10.0 mL. To 1.0 mL of this solution add water to make 50 mL.

(2) Chloride <1.03>—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Sulfate <1.14>—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(4) Heavy metals <1.07>—Proceed with 5.0 g of Maltose Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(5) Arsenic <1.11>—Dissolve 1.5 g of Maltose Hydrate 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, then heat to concentrate to 5 mL, and use this solution as the test solution after cooling. Perform the test (not more than 1.3 ppm).

(6) Dextrin, soluble starch and sulfite—Dissolve 1.0 g of Maltose Hydrate in 10 mL of water, and add 1 drop of iodine TS: a yellow color appears, and the color changes to a blue by adding 1 drop of starch TS.

(7) Nitrogen—Weigh accurately about 2 g of Maltose Hydrate, and perform the test as directed under Nitrogen Determination <1.08> using 10 mL of sulfuric acid for the decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) for the addition: the amount of nitrogen (N: 14.01) is not more than 0.01%.

(8) Related substances—Dissolve 0.5 g of Maltose 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 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 operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of the peaks which appear before the peak of maltose from the sample solution is not larger than 1.5 times the peak area of maltose from the standard solution, and the total area of the peaks which appear after the peak of maltose from the sample solution is not larger than 1/2 times the peak area of maltose 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 sensitivity so that the peak height of maltose obtained from 20 μL of the standard solution is about 30 mm.
Time span of measurement: About 2 times as long as the retention time of maltose.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 4 hours).

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

Assay Weigh accurately about 0.1 g each of Maltose Hydrate and Maltose RS, previously dried, dissolve in exactly 10 mL each of the internal standard solution, 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 operating conditions, and calculate the ratios, Q_T and Q_S, of the peak area of maltose to that of the internal standard.

$$\text{Amount (mg) of maltose hydrate } (C_{12}H_{22}O_{11}.H_2O)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Maltose RS taken

Internal standard solution—A solution of ethylene glycol (1 in 50).
Operating conditions—
Detector: A differential refractometer.
Column: A stainless steel column about 8 mm in inside diameter and about 55 cm in length, packed with gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linking: 8 %) (10 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: Water.
Flow rate: Adjust so that the retention time of maltose is about 18 minutes.
Selection of column: Dissolve 0.25 g of maltose, 0.25 g of glucose and 0.4 g of ethylene glycol in water to make 100 mL. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of maltose, glucose and ethylene glycol in this order with the resolution of between the peaks of maltose and glucose being not less than 4.

Containers and storage Containers—Tight containers.

Freeze-dried Mamushi Antivenom, Equine

乾燥まむしウマ抗毒素

Freeze-dried Mamushi Antivenom, Equine, is a preparation for injection which is dissolved before use.

It contains *Agkistrodon Halys* antivenom in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Mamushi Antivenom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Mamushi Antivenom, Equine, becomes a colorless or light yellow-brown, clear liquid, or a slightly white-turbid liquid on addition of solvent.

Manidipine Hydrochloride

マニジピン塩酸塩
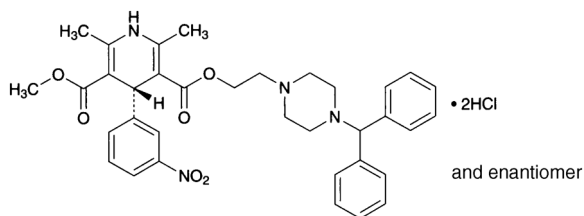
C$_{35}$H$_{38}$N$_4$O$_6$.2HCl: 683.62
3-{2-[4-(Diphenylmethyl)piperazin-1-yl]ethyl}
5-methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-
1,4-dihydropyridine-3,5-dicarboxylate dihydrochloride
[*126229-12-7*]

Manidipine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of manidipine hydrochloride (C$_{35}$H$_{38}$N$_4$O$_6$.2HCl).

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

It is freely soluble in dimethylsulfoxide, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Manidipine Hydrochloride in dimethylsulfoxide (1 in 100) shows no optical rotation.

Manidipine Hydrochloride turns slightly brown-yellowish white on exposure to light.

Melting point: about 207°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Manidipine 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 Manidipine 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 Manidipine Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Manidipine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Add 10 mL of water to 0.1 g of Manidipine Hydrochloride, shake vigorously, and filter. Add 1 drop of ammonia TS to 3 mL of the filtrate, allow to stand 5 minutes, and filter. The filtrate responds to the Qualitative Tests <1.09> (2) for chlorides.

Purity **(1)** Heavy metals <1.07>— Proceed with 1.0 g of Manidipine 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).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Manidipine Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 20 mg of Manidipine Hydrochloride in a mixture of water and acetonitrile (1:1) to make 200 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (1:1) 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 from both solutions by the automatic integration method: the area of the peaks other than manidipine obtained from the sample solution is not larger than 1/5 times the manidipine peak area obtained from the standard solution. Furthermore, the total of the areas of all peaks other than manidipine from the sample solution is not larger than 7/10 times the peak area of manidipine 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 3.5 times as long as the retention time of manidipine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Confirm that the peak area of manidipine obtained from 20 μL of this solution is equivalent to 8 to 12% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 50 mg of Manidipine Hydrochloride in a mixture of water and acetonitrile (1:1) to make 50 mL. To 10 mL of this solution add 5 mL of a solution of butyl benzoate in acetonitrile (7 in 5000) and the mixture of water and acetonitrile (1:1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, manidipine and butyl benzoate 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 manidipine is not more than 2.0%.

Loss on drying <2.41> Not more than 1.5% (1 g, 105°C, 4 hours).

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

Assay Weigh accurately about 0.1 g of Manidipine Hydrochloride, previously dried, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) 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 manidipine to that of the internal standard.

Amount (mg) of manidipine hydrochloride
(C$_{35}$H$_{38}$N$_4$O$_6$.2HCl)
$$= M_S \times Q_T/Q_S \times 4$$

M_S: Amount (mg) of Manidipine Hydrochloride RS taken

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 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 (5 μm in particle diameter).

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

Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 4.6 with diluted potassium hydroxide TS (1 in 10). To 490 mL of this solution add 510 mL of acetonitrile.

Flow rate: Adjust so that the retention time of manidipine 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, manidipine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of manidipine to that of the internal standard is not more than 1.0%.

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

Manidipine Hydrochloride Tablets

マニジピン塩酸塩錠

Manidipine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$: 683.62).

Method of preparation Prepare as directed under Tablets, with Manidipine Hydrochloride.

Identification To a quantity of powdered Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride 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 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 diethylamine (200:1) 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 the standard solution show 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.

Conduct this procedure using light-resistant vessels. To 1 tablet of Manidipine Hydrochloride Tablets, add exactly 1 mL of the internal standard solution per 1 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$), disintegrate by adding a mixture of water and acetonitrile (1:1) to make *V* mL so that each mL contains about 0.1 mg of manidipine hydrochloride

($C_{35}H_{38}N_4O_6 \cdot 2HCl$), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of manidipine hydrochloride
($C_{35}H_{38}N_4O_6 \cdot 2HCl$)
$$= M_S \times Q_T/Q_S \times V/250$$

M_S: Amount (mg) of Manidipine Hydrochloride RS taken

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 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 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 45 minutes of Manidipine Hydrochloride Tablets is not less than 75%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Manidipine 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, and add the dissolution medium to make exactly *V'* mL so that each mL contains about 5.6 μg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$). Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of methanol, 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 manidipine in each solution.

Dissolution rate (%) with respect to the labeled amount of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of Manidipine Hydrochloride RS taken
C: Labeled amount (mg) of manidipine hydrochloride ($C_{35}H_{38}N_4O_6 \cdot 2HCl$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 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 (5 μm in particle diameter).

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

Mobile phase: A mixture of acetonitrile and a solution of potassium dihydrogen phosphate (681 in 100,000) (3:2).

Flow rate: Adjust so that the retention time of manidipine 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 manidipine are not less than 1500 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 manidipine is not more than 2.0%.

Assay   Conduct this procedure using light-resistant vessels. Weigh accurately not less than 20 Manidipine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6.2HCl$), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (1:1) to make 100 mL, shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride RS, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Manidipine Hydrochloride.

$$\text{Amount (mg) of manidipine hydrochloride}$$
$$(C_{35}H_{38}N_4O_6.2HCl)$$
$$= M_S \times Q_T/Q_S \times 2/5$$

M_S: Amount (mg) of Manidipine Hydrochloride RS taken

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).

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

D-Mannitol

D-マンニトール
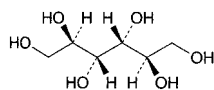
$C_6H_{14}O_6$: 182.17
D-Mannitol
[*69-65-8*]

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 (♦   ♦).

D-Mannitol contains not less than 97.0% and not more than 102.0% of D-mannitol ($C_6H_{14}O_6$), calculated on the dried basis.

♦**Description**   D-Mannitol occurs as white, crystals, powder or grain. It has a sweet taste with a cold sensation.
    It is freely soluble in water, and practically insoluble in ethanol (99.5).
    It dissolves in sodium hydroxide TS.
    It shows crystal polymorphism.♦

Identification   Determine the infrared absorption spectrum of D-Mannitol 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 D-Mannitol RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, put 25 mg each of D-

Mannitol and D-Mannitol RS in glass vessels, dissolve in 0.25 mL of water without heating, dry them in a 600 – 700 W microwave oven for 20 minutes or in a drying chamber at 100°C for 1 hour, then further dry by gradual reducing pressure, and perform the same test as above with so obtained non-sticky white to pale yellow powders: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60>   165 – 170°C

Purity   (1)   Clarity and color of solution—Dissolve 5.0 g of D-Mannitol in water to make 50 mL: the solution is clear, and its clarity is the same as that of water or its turbidity is not more than that of reference suspension I, and its color is not more intense than the following control solution.
    Control solution: To 3.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.4 mL of Copper (II) Sulfate CS, add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.
    ♦(2)   Heavy metals <1.07>—Proceed with 5.0 g of D-Mannitol 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)♦.
    (3)   Nickel—Shake 10.0 g of D-Mannitol with 30 mL of 2 mol/L acetic acid TS, and add water to make exactly 100 mL. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate (about 10 g/L) and 10.0 mL of water-saturated 4-methyl-2-pentanone, and shake for 30 seconds without exposure to light. Allow the layers to separate, and use the 4-methyl-2-pentanone layer as the sample solution. Separately, put 10.0 g each of D-Mannitol in three vessels, add 30 mL of 2 mol/L acetic acid TS to them, shake, add a suitable amount of water and 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 so obtained three 4-methyl-2-pentanone layers as the standard solutions. Additionally, prepare a 4-methyl-2-pentanone layer by proceeding in the same manner as the sample solution without using D-Mannitol, and use this layer as the blank solution. 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. Set the zero of the instrument using the blank solution, and between each measurement, rinse with water and ascertain that the readings return to zero with the blank solution: 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.
    (4)   Related substances—Dissolve 0.50 g of D-Mannitol in water to make 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 0.5 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 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: the peak area of D-sorbitol, having the relative retention time of about 1.2 to D-mannitol, obtained from the sample solution is not larger than that of D-mannitol obtained from the standard solution (1) (not more than 2.0%), the total peak area of maltitol, having the relative retention time of about 0.69, and isomalt,

having the relative retention times of about 0.6 and about 0.73, is not larger than the peak area of D-mannitol from the standard solution (1) (not more than 2.0%), and the area of the peak other than D-mannitol and the peaks mentioned above is not larger than 2 times the peak area of D-mannitol from the standard solution (2) (not more than 0.1%). Furthermore, the total area of the peak other than D-mannitol from the sample solution is not larger than the peak area of D-mannitol from the standard solution (1) (not more than 2.0%). For these calculations exclude the peak which area is not larger than the peak area of D-mannitol from the standard solution (2) (not more than 0.05%).

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 1.5 times as long as the retention time of D-mannitol.

System suitability—

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

◆Test for required detectability: Confirm that the peak area of D-mannitol obtained with 20 μL of the standard solution (2) is equivalent to 1.75 to 3.25% of that obtained with 20 μL of the standard solution (1).

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of D-mannitol is not more than 1.0%.◆

(5) Glucose—To 7.0 g of D-Mannitol add 13 mL of water and 40 mL of Fehling's TS, boil gently for 3 minutes, and allow to stand for 2 minutes to precipitate copper (I) oxide. Separate the supernatant liquid, filter through a sintered glass filter for cupric oxide filtration coated with siliceous earth or a sintered glass filter (G4). Wash the precipitates with 50 – 60°C hot water until the washing no longer alkaline, and filter the washings through the filter described above. Discard all the filtrate at this step. Immediately, dissolve the precipitate with 20 mL of iron (III) sulfate TS, filter through the filter described above in a clean flask, and wash the filter with 15 – 20 mL of water. Combine the filtrate and the washings, heat to 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS until the green color turns to light red and the color persists at least 10 seconds: not more than 3.2 mL is required to change the color of the solution (not more than 0.1% expressed as glucose).

Conductivity <2.51> Dissolve 20.0 g of D-Mannitol in a fleshly boiled and cooled water prepared from distilled water by heating to 40 – 50°C, add the same water to make 100 mL, and use this solution as the sample solution. After cooling, measure the conductivity of the sample solution at 25 ± 0.1°C while gently stirring with a magnetic stirrer: not more than 20 μS·cm^{-1}.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g each of D-Mannitol and D-Mannitol RS (separately determine the loss on drying <2.41> under the same conditions as D-Mannitol), dissolve separately in water to make exactly 10 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 D-mannitol in each solution.

Amount (g) of D-mannitol ($C_6H_{14}O_6$) = $M_S \times A_T/A_S$

M_S: Amount (g) of D-Mannitol RS taken, calculated on the dried basis

Operating conditions—

Detector: A differential refractometer maintained at a constant temperature (40°C for example).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (calcium type) composed with a sulfonated polystyrene cross-linked with 8% of divinylbenzene (9 μm in particle diameter).

Column temperature: 85 ± 2°C.

Mobile phase: water.

Flow rate: 0.5 mL per minute (the retention time of D-mannitol is about 20 minutes).

System suitability—

System performance: Dissolve 0.25 g each of D-Mannitol and D-sorbitol in water to make 10 mL, and use this solution as the solution for system suitability test (1). Separately, dissolve 0.5 g each of maltitol and isomalt in water to make 100 mL. To 2 mL of this solution add water to make 10 mL, and use this solution as the solution for system suitability test (2). When proceed with 20 μL each of the solution for system suitability test (1) and the solution for system suitability test (2) as directed under the above operating conditions, isomalt (first peak), maltitol, isomalt (second peak), D-mannitol and D-sorbitol are eluted in this order, the relative retention time of isomalt (first peak), maltitol, isomalt (second peak) and D-sorbitol to D-mannitol is about 0.6, about 0.69, about 0.73 and about 1.2, respectively, and the resolution between the peaks of D-mannitol and D-sorbitol is not less than 2.0. Coelution of maltitol and the second peak of isomalt may be observed.

◆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 D-mannitol is not more than 1.0%.◆

◆**Containers and storage** Containers—Well-closed containers.◆

D-**Mannitol Injection**

D-**Mannite Injection**

D-マンニトール注射液

D-Mannitol Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of D-mannitol ($C_6H_{14}O_6$: 182.17).

Method of preparation Prepare as directed under Injections, with D-Mannitol. No preservative is added.

Description D-Mannitol Injection is a clear, colorless liquid. It has a sweet taste.

It may precipitate crystals.

Identification Concentrate D-Mannitol Injection on a water bath to make a saturated solution. To 5 drops of this solution add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Shake this solution vigorously: a clear solution is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

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.

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 D-Mannitol Injection, equivalent to about 5 g of D-mannitol ($C_6H_{14}O_6$), and add water to make exactly 250 mL. To exactly 10 mL of this solution add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. After cooling, add 2.5 g of potassium iodide, stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

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

Maprotiline Hydrochloride

マプロチリン塩酸塩
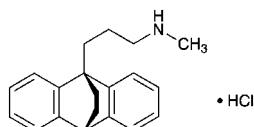
$C_{20}H_{23}N.HCl$: 313.86
3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-
N-methylpropylamine monohydrochloride
[*10347-81-6*]

Maprotiline Hydrochloride, when dried, contains not less than 99.0% of maprotiline hydrochloride ($C_{20}H_{23}N.HCl$).

Description   Maprotiline Hydrochloride occurs as a white crystalline powder.
It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.
Melting point: about 244°C (with decomposition).
It shows crystal polymorphism.

Identification   (1)   Determine the absorption spectrum of a solution of Maprotiline Hydrochloride 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 Maprotiline 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. If any difference ap-

pears between the spectra, recrystallize Maprotiline Hydrochloride with ethanol (99.5), filter, dry the crystals so obtained, and perform the test with the crystals.
(3)   To 5 mL of a solution of Maprotiline Hydrochloride (1 in 200) add 2 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Purity   (1)   Heavy metals <1.07>—Proceed with 2.0 g of Maprotiline 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 0.10 g of Maprotiline Hydrochloride 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 with fluorescent indicator for thin-layer chromatography. Develop with a mixture of 2-butanol, diluted ammonia solution (28) (1 in 3) and ethyl acetate (14:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot 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, 3 hours).

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

Assay   Weigh accurately about 0.25 g of Maprotiline Hydrochloride, previously dried, dissolve in 80 mL of acetic acid (100), add 8 mL of a solution of bismuth nitrate pentahydrate in acetic acid (100) (1 in 50), 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
= 31.39 mg of $C_{20}H_{23}N.HCl$

Containers and storage   Containers—Well-closed containers.

Freeze-dried Live Attenuated Measles Vaccine

乾燥弱毒生麻しんワクチン

Freeze-dried Live Attenuated Measles Vaccine is a preparation for injection which is dissolved before use.
It contains live attenuated measles virus.
It conforms to the requirements of Freeze-dried Live Attenuated Measles Vaccine in the Minimum Requirements for Biological Products.

Description   Freeze-dried Live Attenuated Measles Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Meclofenoxate Hydrochloride

メクロフェノキサート塩酸塩
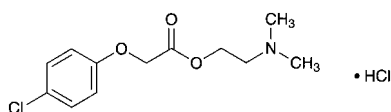
C$_{12}$H$_{16}$ClNO$_3$.HCl: 294.17
2-(Dimethylamino)ethyl (4-chlorophenoxy)acetate monohydrochloride
[*3685-84-5*]

Meclofenoxate Hydrochloride contains not less than 98.0% of meclofenoxate hydrochloride (C$_{12}$H$_{16}$ClNO$_3$. HCl), calculated on the anhydrous basis.

Description Meclofenoxate Hydrochloride occurs as white, crystals or crystalline powder. It has a faint, characteristic odor and a bitter taste.

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

The pH of a solution of 1.0 g of Meclofenoxate Hydrochloride in 20 mL of water is between 3.5 and 4.5.

Identification **(1)** To 10 mg of Meclofenoxate Hydrochloride add 2 mL of ethanol (95), dissolve by warming if necessary, cool, add 2 drops of a saturated solution of hydroxylammonium chloride in ethanol (95) and 2 drops of a saturated solution of potassium hydroxide in ethanol (95), and heat in a water bath for 2 minutes. After cooling, render the solution slightly acidic with dilute hydrochloric acid, and add 3 drops of iron (III) chloride TS: a red-purple to dark purple color develops.

(2) Dissolve 50 mg of Meclofenoxate Hydrochloride in 5 mL of water, and add 2 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Meclofenoxate Hydrochloride (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.

(4) A solution of Meclofenoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 139 – 143°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Meclofenoxate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 1.0 g of Meclofenoxate Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Meclofenoxate 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 Meclofenoxate Hydrochloride according to method 3, and perform the test (not more than 2 ppm).

(5) Organic acids—To 2.0 g of Meclofenoxate Hydrochloride add 50 mL of diethyl ether, shake for 10 minutes, filter through a glass filter (G3), wash the residue with two 5-mL portions of diethyl ether, and combine the washings

with the filtrate. To this solution add 50 mL of neutralized ethanol and 5 drops of phenolphthalein TS, and neutralize with 0.1 mol/L sodium hydroxide VS: the volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.54 mL.

Water <2.48> Not more than 0.50% (1 g, volumetric titration, dirct titration).

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

Assay Weigh accurately about 0.4 g of Meclofenoxate Hydrochloride, dissolve in 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-green through yellow-green to pale greenish yellow [indicator: 3 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)]. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.42 mg of C$_{12}$H$_{16}$ClNO$_3$.HCl

Containers and storage Containers—Tight containers.

Mecobalamin

メコバラミン
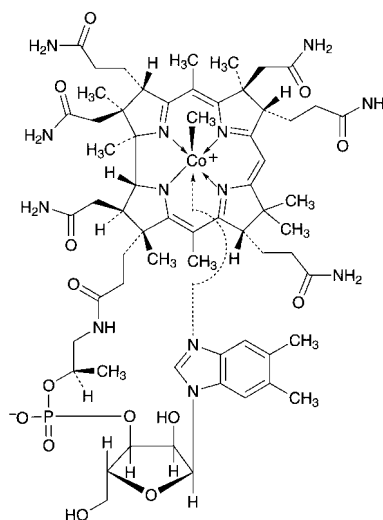
C$_{63}$H$_{91}$CoN$_{13}$O$_{14}$P: 1344.38
*Co*α-[α-(5,6-Dimethyl-1*H*-benzimidazol-1-yl)]-*Co*β-methylcobamide
[*13422-55-4*]

Mecobalamin contains not less than 98.0% and not more than 101.0% of mecobalamin (C$_{63}$H$_{91}$CoN$_{13}$O$_{14}$P), calculated on the anhydrous basis.

Description Mecobalamin occurs as dark red, crystals or crystalline powder.

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

It decomposes on exposure to light.

Identification **(1)** Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of Mecobalamin in hydrochloric acid-potassium chloride buffer solution (pH 2.0) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Refer-

ence Spectrum 1 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Mecobalamin in phosphate buffer solution (pH 7.0) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Mecobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)　Mix 1 mg of Mecobalamin with 50 mg of potassium bisulfate, 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, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate, 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 is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

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

(2)　Related substances—Perform the test with $10 \mu L$ 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: each area of the peaks other than mecobalamin is not more than 0.5% of the peak area of mecobalamin, and the total area of the peaks other than mecobalamin 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 2.5 times as long as the retention time of mecobalamin.

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 the mobile phase 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 the mobile phase to make exactly 10 mL. Confirm that the peak area of mecobalamin obtained from $10 \mu L$ of this solution is equivalent to 7 to 13% of that obtained from $10 \mu L$ of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with $10 \mu L$ of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 3.0%.

Water <2.48>　Not more than 12% (0.1 g, volumetric titration, direct titration).

Assay　Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 50 mg of Mecobalamin and Mecobalamin RS (separately, determine the water <2.48> in the same manner as Mecobalamin), dissolve each 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 10 μL of 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 mecobalamin in each solution.

Amount (mg) of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$)
$= M_S \times A_T/A_S$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 266 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: To 200 mL of acetonitrile add 800 mL of 0.02 mol/L phosphate buffer solution (pH 3.5), then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

Flow rate: Adjust so that the retention time of mecobalamin is about 12 minutes.

System suitability—

System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with $10 \mu L$ of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. And when the procedure is run with $10 \mu L$ of the standard solution under the above operating conditions, the number of theoretical plates of the peak of mecobalamin is not less than 6000.

System repeatability: When the test is repeated 6 times with $10 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 1.0%.

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

Mecobalamin Tablets

メコバラミン錠

Mecobalamin Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$: 1344.38).

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

Identification　**(1)**　Conduct this procedure without exposure to light, using light-resistant vessels. To a quantity of powdered Mecobalamin Tablets, equivalent to 1 mg of Mecobalamin, add 10 mL of hydrochloric acid-potassium chloride buffer solution (pH 2.0), treat with ultrasonic waves, and add hydrochloric acid-potassium chloride buffer solution (pH 2.0) to make 20 mL. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.8 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 262 nm and 266 nm, between 303 nm and 307 nm, and between 461 nm and 465 nm.

(2)　Conduct this procedure without exposure to light, using light-resistant vessels. To a quantity of powdered Mecobalamin Tablets, equivalent to 1 mg of Mecobalamin,

add 10 mL of phosphate buffer solution (pH 7.0), treat with ultrasonic waves, and add phosphate buffer solution (pH 7.0) to make 20 mL. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.8 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 264 nm and 268 nm, between 339 nm and 343 nm, and between 520 nm and 524 nm.

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

Conduct this procedure without exposure to light, using light-resistant vessels. Take 1 tablet of Mecobalamin Tablets, and disintegrate the tablet by adding $V/5$ mL of water. Add methanol to make exactly V mL so that each mL contains about 25 μg of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$). After shaking for 5 minutes, allow to stand for not less than 10 minutes. Filter thus obtained supernatant liquid 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 25 mg of Mecobalamin RS (separately determine the water <2.48> in the same manner as Mecobalamin), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 5 mL of water and methanol to make exactly 50 mL. 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 mecobalamin in each solution.

Amount (mg) of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$)
$$= M_S \times A_T/A_S \times V/1000$$

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

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Mecobalamin.
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 mecobalamin are not less than 2000 and 0.8 to 1.1, 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 mecobalamin 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 Mecobalamin Tablets is not less than 80%.

Conduct this procedure without exposure to light, using light-resistant vessels. Start the test with 1 tablet of Mecobalamin 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 first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 0.28 μg of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$). Use this solution as the sample solution. Separately, weigh accurately about 28 mg of Mecobalamin RS (separately determine the water <2.48> in the same manner as Mecobalamin), and dissolve in 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, 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 mecobalamin in each solution.

Dissolution rate (%) with respect to the labeled amount of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/10$$

M_S: Amount (mg) of Mecobalamin RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 264 nm).
Column: A stainless steel column of 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: Adjust to pH 3.0 of a solution of 6.0 g of L-tartaric acid in 1000 mL of water with a solution of 14.3 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water. To 630 mL of this solution add 370 mL of methanol.
Flow rate: Adjust so that the retention time of mecobalamin is about 8 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 mecobalamin are not less than 3000 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 mecobalamin is not more than 2.0%.

Assay   Conduct this procedure without exposure to light, using light-resistant vessels. Disintegrate 20 tablets of Mecobalamin Tablets with $V/5$ mL of water. Add methanol to make exactly V mL so that each mL contains about 50 μg of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$). After shaking for 5 minutes, allow to stand for not less than 10 minutes. Filter thus obtained supernatant liquid 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 25 mg of Mecobalamin RS (separately determine the water <2.48> in the same manner as Mecobalamin), and dissolve in water to make exactly 100 mL. To exactly 10 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, and determine the peak areas, A_T and A_S, of mecobalamin in each solution.

Amount (mg) of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) in 1 tablet
$$= M_S \times A_T/A_S \times V/10000$$

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

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Mecobalamin.

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 mecobalamin are not less than 3000 and 0.8 to 1.1, respectively.
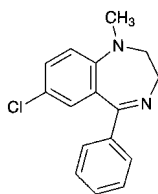
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mecobalamin is not more than 1.0%.

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

Medazepam

メダゼパム

C$_{16}$H$_{15}$ClN$_2$: 270.76
7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-
benzodiazepine
[*2898-12-6*]

Medazepam, when dried, contains not less than 98.5% and not more than 101.0% of medazepam (C$_{16}$H$_{15}$ClN$_2$).

Description   Medazepam occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in methanol, in ethanol (99.5), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It gradually turns yellow on exposure to light.

Identification   (1)   Dissolve 10 mg of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

(2)   Determine the absorption spectrum of a solution of Medazepam 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 Medazepam 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 Medazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60>   101 – 104°C

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Medazepam in 10 mL of methanol: the solution is clear and light yellow to yellow in color.

(2)   Chloride <1.03>—Dissolve 1.5 g of Medazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, and collect the water layer. Wash the water layer with two 20-mL portions of diethyl ether, and filter. To 20 mL of the filtrate add dilute nitric acid to neutralize, 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 0.018%).

(3)   Heavy metals <1.07>—Proceed with 1.0 g of Medazepam 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 Medazepam according to Method 3, and perform the test (not more than 2 ppm).

(5)   Related substances—Dissolve 0.25 g of Medazepam 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 20 mL. Pipet 2 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 cyclohexane, acetone and ammonia solution (28) (60:40:1) 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% (1 g, in vacuum, 60°C, 4 hours).

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

Assay   Weigh accurately about 0.4 g of Medazepam, 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
= 27.08 mg of C$_{16}$H$_{15}$ClN$_2$

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

Medicinal Carbon

薬用炭

Description   Medicinal Carbon occurs as a black, odorless and tasteless powder.

Identification   Place 0.5 g of Medicinal Carbon in a test tube, and heat by direct application of flame with the aid of a current of air: it burns without any flame. Pass the evolved gas through calcium hydroxide TS: a white turbidity is produced.

Purity   (1)   Acidity or alkalinity—Boil 3.0 g of Medicinal Carbon with 60 mL of water for 5 minutes, allow to cool, dilute to 60 mL with water, and filter: the filtrate is colorless and neutral.

(2)   Chloride <1.03>—Take 4.0 mL of the filtrate obtained in (1) in a Nessler tube, add 6 mL of dilute nitric acid and sufficient water to make 50 mL, and 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 0.142%).

(3) Sulfate *<1.14>*—Take 5 mL of the filtrate obtained in (1) in a Nessler tube, add 1 mL of dilute hydrochloric acid and sufficient water to make 50 mL, and 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 0.192%).

(4) Sulfide—Boil 0.5 g of Medicinal Carbon with a mixture of 15 mL of dilute hydrochloric acid and 10 mL of water: lead (II) acetate paper does not become brown when held in the evolved gas within 5 minutes.

(5) Cyanogen compounds—Place a mixture of 5 g of Medicinal Carbon, 2 g of L-tartaric acid and 50 mL of water in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of sodium hydroxide TS and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distil to 25 mL. Dilute the distillate with water to 50 mL. To 25 mL of the diluted distillate add 1 mL of a solution of iron (II) sulfate heptahydrate (1 in 20), heat the mixture almost to boiling, cool, and filter. To the filtrate add 1 mL of hydrochloric acid and 0.5 mL of dilute iron (III) chloride TS: no blue color is produced.

(6) Acid soluble substances—To about 1 g of Medicinal Carbon, accurately weighed, add 20 mL of water and 5 mL of hydrochloric acid, boil for 5 minutes, filter, wash the residue with 10 mL of hot water, and add the washings to the filtrate. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite the residue strongly: the mass of the residue is not more than 3.0%.

(7) Heavy metals *<1.07>*—Proceed with 0.5 g of Medicinal Carbon 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).

(8) Zinc—Ignite 0.5 g of Medicinal Carbon to ash, add 5 mL of dilute nitric acid to the residue, boil gently for 5 minutes, filter, wash with 10 mL of water, and combine the washings and the filtrate. Add 3 mL of ammonia TS to the solution, filter again, wash with water, combine the washings and the filtrate, add another washing to make 25 mL, add 1 drop of sodium sulfide TS, and allow to stand for 3 minutes: the liquid produces no turbidity.

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

Loss on drying *<2.41>* Not more than 15.0% (1 g, 105°C, 4 hours).

Residue on ignition *<2.44>* Not more than 4% (1 g).

Adsorptive power **(1)** Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of quinine sulfate hydrate, shake the mixture vigorously for 5 minutes, filter immediately, and reject the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

(2) Dissolve 250 mg of methylene blue trihydrate, exactly weighed, in water to make exactly 250 mL. Measure two 50-mL portions of this solution into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihy-

drate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate *<2.50>* the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS. The difference between the two titrations is not less than 1.2 mL.

Containers and storage Containers—Well-closed containers.

Medicinal Soap

薬用石ケン

Medicinal Soap is sodium salts of fatty acids.

Description Medicinal Soap occurs as white to light yellow, powder or granules. It has a characteristic odor free from rancidity.

Medicinal Soap is sparingly soluble in water, and slightly soluble in ethanol (95).

A solution of Medicinal Soap (1 in 100) is alkaline.

Fatty acid Dissolve 25 g of Medicinal Soap in 300 mL of hot water, add 60 mL of dilute sulfuric acid slowly, and warm in a water bath for 20 minutes. After cooling, filter off the precipitate, and wash with warm water until the washing no longer shows acidity to methyl orange TS. Transfer the precipitate to a small beaker, and heat on a water bath to complete separation of water and transparent fatty acids. Filter the fatty acid into a small beaker while warm, dry at 100°C for 20 minutes, and perform the test with this material as directed under Fats and Fatty Oils *<1.13>*. The congealing point of the fatty acid is between 18°C and 28°C. The acid value is 185 – 205. The iodine value is 82 – 92.

Purity **(1)** Acidity or alkalinity—Dissolve 5.0 g of Medicinal Soap in 85 mL of neutralized ethanol by warming on a water bath, filter while hot through absorbent cotton, and wash the filter and the residue with three 5-mL portions of hot neutralized ethanol. Combine the filtrate and the washings, add hot neutralized ethanol to make exactly 100 mL, and perform the following tests quickly using this as the sample solution at 70°C.

(i) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS to 40 mL of the sample solution: a red color develops.

(ii) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS to 40 mL of the sample solution: no red color develops.

(2) Heavy metals *<1.07>*—Proceed with 1.0 g of Medicinal Soap 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) Ethanol-insoluble substances—Weigh accurately about 2 g of Medicinal Soap, dissolve by warming in 100 mL of neutralized ethanol, filter the solution through a glass filter (G4), wash the residue with 100 mL of hot neutralized ethanol, and dry at 105°C for 4 hours: the mass of the residue is not more than 1.0%.

(4) Water-insoluble substances—Wash thoroughly the dried substances obtained in (3) with 200 mL of water, and dry at 105°C for 4 hours: the mass of the residue is not more than 0.15%.

(5) Alkali carbonates—To the washings obtained in (4) add 3 drops of methyl orange TS and 2 mL of 0.05 mol/L sulfuric acid VS: a red color develops.

Loss on drying   Not more than 5.0% in the case of the powder, and not more than 10.0% in the case of the granules.

Weigh accurately about 0.5 g of Medicinal Soap in a tared beaker, add 10 g of sea sand (No. 1), previously dried at 105°C for 1 hour, and again weigh the beaker. Add 10 mL of ethanol (95), evaporate on a water bath to dryness with thorough stirring, and dry at 105°C for 3 hours.

Containers and storage   Containers—Well-closed containers.

Medroxyprogesterone Acetate

メドロキシプロゲステロン酢酸エステル
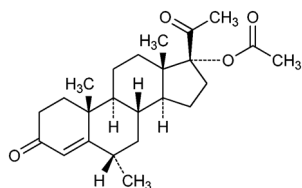
C$_{24}$H$_{34}$O$_{4}$: 386.52
6α-Methyl-3,20-dioxopregn-4-en-17-yl acetate
[*71-58-9*]

Medroxyprogesterone Acetate, when dried, contains not less than 97.0 and not more than 103.0% of medroxyprogesterone acetate (C$_{24}$H$_{34}$O$_{4}$).

Description   Medroxyprogesterone Acetate occurs as a white crystalline powder.

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

Identification   (1)   Determine the absorption spectrum of a solution of Medroxyprogesterone Acetate 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 Medroxyprogesterone Acetate 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 Medroxyprogesterone 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 dried Medroxyprogesterone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49>   [α]$_{D}^{20}$: + 47 – + 53°(after drying, 0.25 g, acetone, 25 mL, 100 mm).

Melting point <2.60>   204 – 209°C

Purity   (1)   Heavy metals <1.07>—Proceed with 1.0 g of Medroxyprogesterone Acetate 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—Use the sample solution obtained in the Assay 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 area of the peak other than medroxyprogesterone acetate obtained from the sample solution is not larger than the peak area of medroxyprogesterone acetate obtained from the standard solution, and the total area of the peaks other than medroxyprogesterone acetate from the sample solution is not larger than 2 times the peak area of medroxyprogesterone acetate 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 1.2 times as long as the retention time of medroxyprogesterone acetate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of medroxyprogesterone acetate 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: 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 medroxyprogesterone acetate 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, the relative standard deviation of the peak area of medroxyprogesterone acetate is not more than 2.0%.

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).

Assay   Weigh accurately about 25 mg each of Medroxyprogesterone Acetate and Medroxyprogesterone Acetate RS, both previously dried, dissolve in acetonitrile to make them exactly 25 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 medroxyprogesterone acetate in each solution.

Amount (mg) of medroxyprogesterone acetate (C$_{24}$H$_{34}$O$_{4}$)
   = M_{S} × A_{T}/A_{S}

M_{S}: Amount (mg) of Medroxyprogesterone Acetate RS taken

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 water and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of medroxyprogesterone acetate is about 31 minutes.

System suitability—

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 medroxyprogesterone acetate 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, the relative standard deviation of the peak area of medroxyprogesterone acetate is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Mefenamic Acid

メフェナム酸
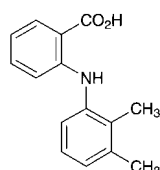
C$_{15}$H$_{15}$NO$_2$: 241.29
2-(2,3-Dimethylphenylamino)benzoic acid
[*61-68-7*]

Mefenamic Acid, when dried, contains not less than 99.0% of mefenamic acid (C$_{15}$H$_{15}$NO$_2$).

Description Mefenamic Acid occurs as a white to light yellow powder. It is odorless and tasteless at first, but leaves a slightly bitter aftertaste.

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

It dissolves in sodium hydroxide TS.

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

Identification **(1)** Dissolve 10 mg of Mefenamic Acid in 1 mL of methanol by warming, cool, add 1 mL of a solution of 4-nitrobenzene diazonium fluoroborate (1 in 1000) and 1 mL of sodium hydroxide TS, and mix thoroughly: an orange-red color is produced.

(2) Dissolve 10 mg of Mefenamic Acid in 2 mL of sulfuric acid, and heat: the solution shows a yellow color and a green fluorescence.

(3) Dissolve 7 mg of Mefenamic Acid in a solution of hydrochloric acid in methanol (1 in 1000) 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.

Purity **(1)** Chloride <1.03>—To 1.0 g of Mefenamic Acid add 20 mL of sodium hydroxide TS, and dissolve by warming. Cool, add 2 mL of acetic acid (100) and water to make 100 mL, and mix well. Remove the produced precipitate by filtration, discard the first 10 mL of the filtrate, and to subsequent 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 as follows: to 0.50 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 0.5 mL of acetic acid (100), 6 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Mefenamic Acid 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 Mefenamic Acid according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Mefenamic Acid, in 5 mL of a mixture of chloroform and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (3:1) to make exactly 200 mL, pipet 10 mL of this solution, add a mixture of chloroform and methanol (3:1) 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 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 2-methyl-1-propanol and ammonia solution (28) (3:1) 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% (1 g, in vacuum, 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 Mefenamic Acid, previously dried, and dissolve in 100 mL of ethanol (95), previously neutralized to phenol red TS with 0.1 mol/L sodium hydroxide VS, by warming gently. Cool, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through yellow-red to red-purple (indicator: 2 to 3 drops of phenol red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.13 mg of C$_{15}$H$_{15}$NO$_2$

Containers and storage Containers—Well-closed containers.

Mefloquine Hydrochloride

メフロキン塩酸塩
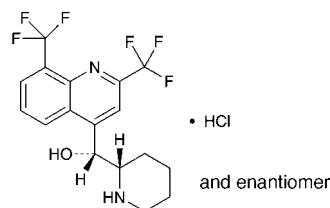
C$_{17}$H$_{16}$F$_6$N$_2$O.HCl: 414.77
(1*RS*)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol monohydrochloride
[*51773-92-3*]

Mefloquine Hydrochloride contains not less than 99.0% and not more than 101.0% of mefloquine hydrochloride (C$_{17}$H$_{16}$F$_6$N$_2$O.HCl), calculated on the anhydrous basis.

Description Mefloquine Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5),

and slightly soluble in water.

It dissolves in sulfuric acid.

A solution of Mefloquine Hydrochloride in methanol (1 in 20) shows no optical rotation.

Melting point: about 260°C (with decomposition).

Identification (1) Dissolve 2 mg of Mefloquine Hydrochloride in 1 mL of sulfuric acid: the solution shows a blue fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Mefloquine Hydrochloride in methanol (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 Mefloquine Hydrochloride, previously dried at 105°C for 2 hours, 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) To 5 mL of a solution of Mefloquine Hydrochloride (1 in 1000) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is formed, and the separated precipitate dissolves on the addition of an excess amount of ammonia TS.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Mefloquine Hydrochloride according to Method 2 using a quartz crucible, 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>—To 1.0 g of Mefloquine Hydrochloride add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, gradually heat, and incinerate by ignition at 800°C. If a carbonized residue still retains, moisten the residue with a little amount of nitric acid, and ignite again to incinerate. After cooling, to the residue add 3 mL of hydrochloric acid, warm on a water bath to dissolve, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Mefloquine Hydrochloride 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 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 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 mefloquine and the peak eluted first from the sample solution is not larger than the peak area of mefloquine from the standard solution, and the total area of the peaks other than the peak of mefloquine and the peak eluted first from the sample solution is not larger than 2.5 times the peak area of mefloquine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with aminopropylsilanized 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 and diluted phosphoric acid (1 in 14) (24:1).

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

Time span of measurement: About 3 times as long as the retention time of mefloquine.

System suitability—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of mefloquine 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: Dissolve 10 mg of mefloquine hydrochloride and 5 mg of diprophylline in 50 mL of the mobile phase. 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, diprophylline and mefloquine 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 area of mefloquine is not more than 2.0%.

Water <2.48> Not more than 3.0% (1 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.5 g of Mefloquine Hydrochloride, 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 a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 41.48 \text{ mg of } C_{17}H_{16}F_6N_2O \cdot HCl$$

Containers and storage Containers—Well-closed containers.

Mefruside

メフルシド
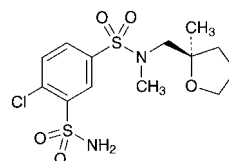
and enantiomer

$C_{13}H_{19}ClN_2O_5S_2$: 382.88
4-Chloro-*N*-methyl-*N*-[(2*RS*)-2-methyltetrahydrofuran-2-ylmethyl]-3-sulfamoylbenzenesulfonamide
[*7195-27-9*]

Mefruside, when dried, contains not less than 98.5% of mefruside ($C_{13}H_{19}ClN_2O_5S_2$).

Description Mefruside occurs as a white crystalline powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

A solution of Mefruside in *N*,*N*-dimethylformamide (1 in 10) has no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Mefruside 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 Mefruside, 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 Mefruside as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 149 – 152°C

Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Mefruside 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).

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

(3) Related substances—Dissolve 0.20 g of Mefruside in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone 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 and acetone (5:2) 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.

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 0.5 g of Mefruside, previously dried, dissolve in 80 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 13 mL of water to 80 mL of N,N-dimethylformamide, and make any necessary correction.

> Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
> = 38.29 mg of $C_{13}H_{19}ClN_2O_5S_2$

Containers and storage Containers—Well-closed containers.

Mefruside Tablets

メフルシド錠

Mefruside Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$: 382.88).

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

Identification (1) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.3 g of Mefruside, shake with 15 mL of heated methanol for 20 minutes, and filter. Add 25 mL of water to the filtrate, and allow to stand while ice-cooling for 30 minutes. Filter the white precipitate formed, wash with water, and dry at 105°C for 2 hours: the precipitate melts <2.60> between 149°C and 152°C.

(2) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.01 g of Mefruside, shake with 70 mL of methanol strongly for 15 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 274 nm and 278 nm, and 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 tablet of Mefruside Tablets add 40 mL of methanol, disintegrate the tablet using ultrasonic waves with occasional stirring, then further treat with ultrasonic waves for 10 minutes, and add methanol to make exactly V mL of a solution containing about 0.5 mg of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) per mL. Centrifuge the solution, pipet 5 mL of the supernatant liquid, add methanol to make exactly 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

> Amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)
> = $M_S \times A_T/A_S \times V/125$

M_S: Amount (mg) of mefruside 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 Mefruside Tablets is not less than 85%.

Start the test with 1 tablet of Mefruside Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a filter paper for quantitative analysis (5C). Discard the first 5 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 mefruside ($C_{13}H_{19}ClN_2O_5S_2$), and use this solution as the sample solution. Separately, weigh accurately about 70 mg of mefruside for assay, previously dried at 105°C for 2 hours, dissolve in methanol 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, of the sample solution and standard solution at 285 nm in a layer of 5 cm in length as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

> Dissolution rate (%) with respect to the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)
> = $M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of mefruside for assay taken
C: Labeled amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) in 1 tablet

Assay Weigh accurately not less than 20 Mefruside Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 65 mg of mefruside ($C_{13}H_{19}ClN_2O_5S_2$), shake with 70 mL of methanol for 15 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly 10

mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 65 mg of mefruside for assay, previously dried at 105°C for 2 hours, and dissolve in 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 285 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of mefruside } (C_{13}H_{19}ClN_2O_5S_2)$$
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of mefruside for assay taken

Containers and storage   Containers—Tight containers.

Meglumine

メグルミン
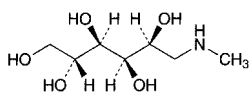
C$_7$H$_{17}$NO$_5$: 195.21
1-Deoxy-1-methylamino-D-glucitol
[*6284-40-8*]

Meglumine, when dried, contains not less than 99.0% of meglumine (C$_7$H$_{17}$NO$_5$).

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

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

The pH of a solution of 1.0 g of Meglumine in 10 mL of water is between 11.0 and 12.0.

Identification   (1)   To 1 mL of a solution of Meglumine (1 in 10) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS: a deep red color develops.

(2)   To 2 mL of a solution of Meglumine (1 in 10) add 1 drop of methyl red TS, and add 0.5 mL of dilute sodium hydroxide TS and 0.5 g of boric acid after neutralizing with 0.5 mol/L sulfuric acid TS: a deep red color develops.

(3)   Dissolve 0.5 g of Meglumine in 1 mL of diluted hydrochloric acid (1 in 3), and add 10 mL of ethanol (99.5): a white precipitate is produced. Then, rubbing the inside wall of the container with a glass rod, cool with ice and produce more precipitate. Filter the precipitate by suction through a glass filter (G3), wash the precipitate with a small volume of ethanol (99.5), and dry at 105°C for 1 hour: the residue thus obtained melts <2.60> between 149°C and 152°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-16.0 - -17.0°$ (after drying, 1 g, water, 10 mL, 100 mm).

Melting point <2.60>   128 – 131°C

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Meglumine in 10 mL of water: the solution is clear and colorless.

(2)   Chloride <1.03>—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 10 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 0.009%).

(3)   Sulfate <1.14>—Dissolve 1.0 g of Meglumine in 30

mL of water, and add 5 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 0.019%).

(4)   Heavy metals <1.07>—Proceed with 2.0 g of Meglumine 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 2.0 g of Meglumine according to Method 3, and perform the test (not more than 1 ppm).

(6)   Reducing substances—To 5 mL of a solution of Meglumine (1 in 20) add 5 mL of Fehling's TS, and boil for 2 minutes: no red-brown precipitate is produced.

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 Meglumine, previously dried, dissolve in 25 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 19.52 mg of C$_7$H$_{17}$NO$_5$

Containers and storage   Containers—Tight containers.

Meglumine Iotalamate Injection

イオタラム酸メグルミン注射液

Meglumine Iotalamate Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iotalamic acid (C$_{11}$H$_9$I$_3$N$_2$O$_4$: 613.91).

Method of preparation

(1)

Iotalamic Acid	227.59 g
Meglumine	72.41 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

(2)

Iotalamic Acid	455 g
Meglumine	145 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

Description   Meglumine Iotalamate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification   (1)   To 1 mL of Meglumine Iotalamate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(2)   To a volume of Meglumine Iotalamate Injection, equivalent to 1 g of Iotalamic Acid, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid while shaking: a

white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 4 hours. Proceed with the precipitate so obtained as directed in the Identification (2) under Iotalamic Acid.

Optical rotation <2.49>
 Method of preparation (1) α_D^{20}: $-1.67 - -1.93°$ (100 mm).
 Method of preparation (2) α_D^{20}: $-3.35 - -3.86°$ (100 mm).

pH <2.54> 6.5 – 7.7

Purity **(1)** Primary aromatic amines—To a volume of Meglumine Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—Take a volume of Meglumine Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid, add 20 mL of water and 5 mL of dilute sulfuric acid, shake well, and filter the precipitate by suction throuth a glass filter (G4). To the filtrate add 5 mL of toluene, and shake vigorously: the toluene layer is colorless. Then add 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously: the toluene layer has no more color than the following control solution.

Control solution: Dissolve 0.25 g of potassium iodide in water to make 1000 mL. To 2.0 mL of this solution add 20 mL of water, 5 mL of dilute sulfuric acid, 5 mL of toluene and 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously.

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 Meglumine Iotalamate Injection, equivalent to about 4 g of iotalamic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 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 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 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 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 iotalamic acid to that of the internal standard.

$$\text{Amount (mg) of iotalamic acid } (C_{11}H_9I_3N_2O_4)$$
$$= M_S \times Q_T/Q_S \times 10$$

M_S: Amount (mg) of iotalamic acid for assay taken

Internal standard solution—A solution of L-tryptophan in

the mobile phase (3 in 2500).
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 20°C.
 Mobile phase: Dissolve 3.9 g of phosphoric acid and 2.8 mL of triethylamine in water to make 2000 mL. To this solution add 100 mL of acetonitrile.
 Flow rate: Adjust so that the retention time of iotalamic acid 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, iotalamic acid 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 ratios of the peak area of iotalamic acid 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.

Meglumine Sodium Amidotrizoate Injection

アミドトリゾ酸ナトリウムメグルミン注射液

 Meglumine Sodium Amidotrizoate Injection is an aqueous injection.
 It contains not less than 95.0% and not more than 105.0% of the labeled amount of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

Method of preparation

(1)	
Amidotrizoic Acid (anhydrous)	471.78 g
Sodium Hydroxide	5.03 g
Meglumine	125.46 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

(2)	
Amidotorizoic Acid (anhydrous)	597.30 g
Sodium Hydroxide	6.29 g
Meglumine	159.24 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

 Prepare as directed under Injections, with the above ingredients (1) or (2).

Description Meglumine Sodium Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.
 It gradually changes in color by light.

Identification **(1)** To a volume of Meglumine Sodium

Amidotrizoate Injection, equivalent to 1 g of Amidotrizoic Acid, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

(2) To 1 mL of Meglumine Sodium Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(3) Meglumine Sodium Amidotrizoate Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49>
Method of preparation (1) α_D^{20}: $-2.91 - -3.36°$ (100 mm).
Method of preparation (2) α_D^{20}: $-3.69 - -4.27°$ (100 mm).

pH <2.54>   6.0 – 7.7

Purity (1) Primary aromatic amines—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.20 g of Amidotrizoic Acid, add 6 mL of water, mix, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, and shake. Proceed as directed in the Purity (2) under Amidotrizoic Acid: the absorbance is not more than 0.19.

(2) Iodine and iodide—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.25 g of Amidotrizoic Acid, add water to make 20 mL, add 5 mL of dilute nitric acid, shake well, and filter by suction through a glass filter (G4). Add 5 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of hydrogen peroxide (30), and shake vigorously: the chloroform layer has no more color than the following control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. Add 20 mL of water to 0.10 mL of this solution, add 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of hydrogen peroxide (30), and shake vigorously.

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 Meglumine Sodium Amidotrizoate Injection, equivalent to about 0.5 g of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of amidotrizoic acid for assay (separately determine the loss on drying <2.41> under the same condition as Amidotrizoic Acid), dissolve in a solution of meglumine (3 in 1000) 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 100 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 amidotrizoic acid to that of the internal standard.

$$\text{Amount (mg) of amidotrizoic acid } (C_{11}H_9I_3N_2O_4)$$
$$= M_S \times Q_T/Q_S \times 2$$

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

Internal standard solution—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL.
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 1.7 g of tetrabutylammonium dihydrogen phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.
Flow rate: Adjust so that the retention time of amidotrizoic acid 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, amidotrizoic acid 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid 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.

Melphalan

メルファラン
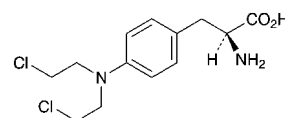
$C_{13}H_{18}Cl_2N_2O_2$: 305.20
4-Bis(2-chloroethyl)amino-L-phenylalanine
[*148-82-3*]

Melphalan contains not less than 93.0% of melphalan ($C_{13}H_{18}Cl_2N_2O_2$), calculated on the dried basis.

Description   Melphalan occurs as a white to light yellowish white crystalline powder.
It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.
It dissolves in dilute hydrochloric acid and in dilute sodium hydroxide TS.
It is gradually colored by light.
Optical rotation $[\alpha]_D^{20}$: about $-32°$ (0.5 g calculated on the dried basis, methanol, 100 mL, 100 mm).

Identification   (1)   To 20 mg of Melphalan add 50 mL of

methanol, dissolve by warming, add 1 mL of a solution of 4-(4-nitrobenzyl)pyridine in acetone (1 in 20), and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of warmed methanol and add 2 drops of ammonia solution (28): a purple color develops.

(2) Dissolve 0.1 g of Melphalan in 10 mL of dilute sodium hydroxide TS, and heat on a water bath for 10 minutes. After cooling, add dilute nitric acid to acidify, and filter: the filtrate responds to the Qualitative Tests <1.09> for chloride.

(3) Determine the absorption spectrum of a solution of Melphalan 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.

Purity **(1)** Ionisable chloride—Weigh accurately about 0.5 g of Melphalan, dissolve in 80 mL of diluted nitric acid (1 in 40), stir for 2 minutes, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration): the consumed volume is not more than 1.0 mL to 0.50 g of Melphalan.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Melphalan 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 Melphalan according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41>   Not more than 7.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 105°C, 2 hours).

Residue on ignition <2.44>   Not more than 0.3% (1 g).

Assay Weigh accurately about 0.25 g of Melphalan, add 20 mL of a solution of potassium hydroxide (1 in 5), and heat under a reflux condenser on a water bath for 2 hours. After cooling, add 75 mL of water and 5 mL of nitric acid, cool, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Make any necessary correction by using the results obtained in the Purity (1).

Each mL of 0.1 mol/L silver nitrate VS
$$= 15.26 \text{ mg of } C_{13}H_{18}Cl_2N_2O_2$$

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

Menatetrenone

メナテトレノン
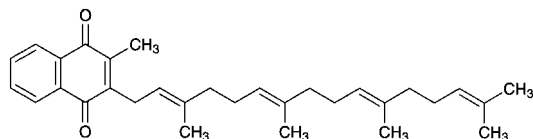
$C_{31}H_{40}O_2$: 444.65
2-Methyl-3-[(2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]-1,4-naphthoquinone
[*863-61-6*]

Menatetrenone contains not less than 98.0% of menatetrenone ($C_{31}H_{40}O_2$), calculated on the anhydrous basis.

Description Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by light.

Melting point: about 37°C.

Identification **(1)** Dissolve 0.1 g of Menatetrenone in 5 mL of ethanol (99.5) by warming, cool, and add 1 mL of a solution of potassium hydroxide in ethanol (95) (1 in 10): a blue color develops, and upon standing it changes from blue-purple to red-brown through red-purple.

(2) Determine the infrared absorption spectrum of Menatetrenone, after melting by warming if necessary, as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Menatetrenone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Menatetrenone 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) Menadione—To 0.20 g of Menatetrenone add 5 mL of diluted ethanol (1 in 2), shake well, and filter. To 0.5 mL of the filtrate add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazorone in ethanol (99.5) (1 in 20) and 1 drop of ammonia water (28), and allow to stand for 2 hours: no blue-purple color develops.

(3) *cis* Isomer—Dissolve 0.10 g of Menatetrenone in 10 mL of hexane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane 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 chromatogram with a mixture of hexane and di-*n*-butyl ether (17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot corresponding to relative *R*f value 1.1 regarding to the principal spot from the sample solution is not more intense than the spot from the standard solution.

(4) Related substances—Conduct this procedure without exposure to light, using a light-resistant vessel. Dissolve 0.10 g of Menatetrenone in 100 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 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 total area of peaks other than the peak of menatetrenone from the sample solution is not larger than the peak area of menatetrenone 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 6 times as long as the retention time of menatetrenone, 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 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL.

Confirm that the peak area of menatetrenone obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 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 areas of menatetrenone is not more than 1.0%.

Water <2.48>   Not more than 0.5% (0.5 g, volumetric titration, direct titration).

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

Assay   Conduct this procedure without exposure to light, using a light-resistant vessel. Weigh accurately about 0.1 g each of Menatetrenone and Menatetrenone RS (separately, determine the water <2.48> in the same manner as Menatetrenone), dissolve each in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of these solutions, and add ethanol (99.5) to make exactly 100 mL. Pipet 2 mL each of these solutions, add exactly 4 mL each of the internal standard solution, 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 menatetrenone to that of the internal standard.

Amount (mg) of menatetrenone $(C_{31}H_{40}O_2) = M_S \times Q_T/Q_S$

M_S: Amount (mg) of Menatetrenone RS taken, calculated on the dehydrated basis

Internal standard solution—A solution of phytonadione in 2-propanol (1 in 20,000).
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 40°C.
Mobile phase: Methanol.
Flow rate: Adjust so that the retention time of menatetrenone 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, menatetrenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
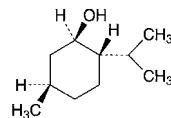System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of menatetrenone to that of the internal standard is not more than 1.0%.

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

dl-Menthol

dl-メントール

and enantiomer

$C_{10}H_{20}O$: 156.27
(1RS,2SR,5RS)-5-Methyl-2-(1-methylethyl)cyclohexanol
[*89-78-1*]

dl-Menthol contains not less than 98.0% of *dl*-menthol $(C_{10}H_{20}O)$.

Description   *dl*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

Identification   **(1)**   Triturate *dl*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2)   Shake 1 g of *dl*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer possesses no aroma of menthol is separated.

Congealing point <2.42>   27 – 28°C

Optical rotation <2.49> $[\alpha]_D^{20}$: −2.0 – +2.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Purity   **(1)**   Non-volatile residue—Volatilize 2.0 g of *dl*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2)   Thymol—Add 0.20 g of *dl*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3)   Nitromethane or nitroethane—To 0.5 g of *dl*-Menthol placed in a flask add 2 mL of a solution of sodium hydroxide (1 in 2) and 1 mL of hydrogen peroxide (30), connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, then add 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N,N*-diethyl-*N′*-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

Assay   Weigh accurately about 2 g of *dl*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash down the condenser with 20 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.
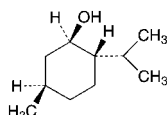
Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of $C_{10}H_{20}O$

Containers and storage   Containers—Tight containers.

Storage—In a cold place.

l-Menthol

l-メントール

C$_{10}$H$_{20}$O: 156.27
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol
[*2216-51-5*]

l-Menthol contains not less than 98.0% of *l*-menthol (C$_{10}$H$_{20}$O).

Description   *l*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

Identification (1)   Triturate *l*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2)   Shake 1 g of *l*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer which possesses no aroma of menthol is separated.

Optical rotation *<2.49>*   [α]$_D^{20}$: −45.0 – −51.0°   (2.5 g, ethanol (95), 25 mL, 100 mm).

Melting point *<2.60>*   42 – 44°C

Purity (1)   Non-volatile residue—Volatilize 2.0 g of *l*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2)   Thymol—Add 0.20 g of *l*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3)   Nitromethane or nitroethane—To 0.5 g of *l*-Menthol placed in a flask add 2 mL of a solution of sodium hydroxide (1 in 2) and 1 mL of hydrogen peroxide (30), connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, add 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N*,*N*-diethyl-*N*′-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

Assay   Weigh accurately about 2 g of *l*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash the condenser with 20 mL of water, and titrate *<2.50>* with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
    = 156.3 mg of C$_{10}$H$_{20}$O

Containers and storage   Containers—Tight containers.
Storage—In a cold place.

Mepenzolate Bromide

メペンゾラート臭化物
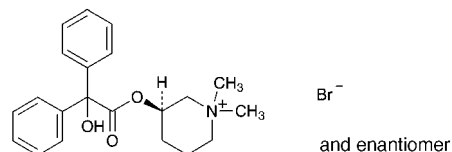
C$_{21}$H$_{26}$BrNO$_3$: 420.34
(3*RS*)-3-[(Hydroxy)(diphenyl)acetoxy]-1,1-dimethylpiperidinium bromide
[*76-90-4*]

Mepenzolate Bromide, when dried, contains not less than 98.5% of mepenzolate bromide (C$_{21}$H$_{26}$BrNO$_3$).

Description   Mepenzolate Bromide is white to pale yellow, crystals or crystalline powder. It is odorless, and has a bitter taste.

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

Melting point: about 230°C (with decomposition).

Identification (1)   To 30 mg of Mepenzolate Bromide add 10 drops of sulfuric acid: a red color develops.

(2)   Dissolve 10 mg of Mepenzolate Bromide in 20 mL of water and 5 mL of dilute hydrochloric acid, and to 5 mL of this solution add 1 mL of Dragendorff's TS: an orange precipitate is produced.

(3)   Determine the absorption spectrum of a solution of Mepenzolate Bromide in 0.01 mol/L hydrochloric acid TS (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.

(4)   Dissolve 0.5 g of Mepenzolate Bromide in 50 mL of water and 3 mL of nitric acid by heating. This solution responds to the Qualitative Tests *<1.09>* for Bromide.

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

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

(3)   Related substances—Dissolve 0.40 g of Mepenzolate Bromide in exactly measured 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 (1). Separately, dissolve 40 mg of benzophenone 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 (2). Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 10 μL each of the sample solution, standard solutions (1) and (2) on a plate of silica gel with fluorecent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, 1-butanol, water and acetic acid (100) (3:3:2:1) to a distance of

about 10 cm, and air-dry the plate and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than either the principal spot or the spot corresponding to benzophenone from the sample solution are not more intense than the spot from standard solution (1), and the spot corresponding to benzophenone from the sample solution is not more intense than the spot from standard solution (2). Spray evenly Dragendorff's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from standard solution (1).

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 Mepenzolate Bromide, previously dried, dissolve in 2 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
= 42.03 mg of $C_{21}H_{26}BrNO_3$

Containers and storage   Containers—Tight containers.

Mepitiostane

メピチオスタン
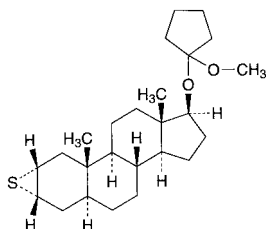
$C_{25}H_{40}O_2S$: 404.65
$2\alpha,3\alpha$-Epithio-17β-(1-methoxycyclopentyloxy)-5α-androstane
[*21362-69-6*]

Mepitiostane contains not less than 96.0% and not more than 102.0% of mepitiostane ($C_{25}H_{40}O_2S$), calculated on the anhydrous basis.

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

It is freely soluble in triethylamine, in chloroform, in diethyl ether and in cyclohexane, soluble in diethylene glycol dimethyl ether and in petroleum ether, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is hydrolyzed in moist air.

Identification   (1)   Dissolve 1 mg of Mepitiostane in 1 mL of methanol, and add 0.5 mL of palladium (II) chloride TS: an orange precipitate is formed. To this suspension add 1 mL of water and 2 mL of chloroform, shake well, and allow to stand: an orange color develops in the chloroform layer.

(2)   Dissolve 0.1 g of Mepitiostane in 2 mL of diethylene glycol dimethyl ether, shake with 1 mL of 1 mol/L hydrochloric acid TS, and filter. To the filtrate add 1.5 mL of 2,4-dinitrophenylhydrazine-diethylene glycol dimethyl ether TS and 1.5 mL of diluted ethanol (95) (2 in 3): an orange-yellow precipitate is formed. Filter the precipitate, recrystallize

from ethanol (99.5), and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the crystals melt <2.60> between 144°C and 149°C.

(3)   Determine the infrared absorption spectrum of Mepitiostane 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> $[\alpha]_D^{20}$: +20 – +23° (0.1 g, chloroform, 10 mL, 100 mm).

Purity   (1)   Clarity and color of solution—Dissolve 0.10 g of Mepitiostane in 4 mL of petroleum ether: the solution is clear and colorless to pale yellow.

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Mepitiostane 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 Mepitiostane in exactly 5 mL of a mixture of acetone and triethylamine (1000:1), and use this solution as the sample solution. Separately, dissolve 10 mg of Epitiostanol RS in a mixture of acetone and triethylamine (1000:1) to make exactly 10 mL. Pipet 1 mL and 3 mL of this solution, to each add a mixture of acetone and triethylamine (1000:1) to make exactly 25 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. 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 fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, heat between 120°C and 130°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution showing the same Rf value as the standard solutions are not more intense than the spot from the standard solution (2), and the remaining spots other than the principal spot are not more intense than the spot from the standard solution (1).

Water <2.48>   Not more than 0.7% (0.3 g, volumetric titration, back titration).

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

Assay   Weigh accurately about 0.3 g of Mepitiostane, and dissolve in cyclohexane to make exactly 10 mL. Pipet 2 mL of this solution, add 10 mL of ethanol (99.5), mix with exactly 2 mL each of 0.01 mol/L hydrochloric acid TS and the internal standard solution, add ethanol (99.5) to make 20 mL, allow to stand at ordinary temperature for 30 minutes, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Epitiostanol RS, dissolve in exactly 2 mL of the internal standard solution, add ethanol (99.5) 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 epitiostanol to that of the internal standard, respectively.

Amount (mg) of mepitiostane ($C_{25}H_{40}O_2S$)
$= M_S \times Q_T/Q_S \times 5 \times 1.320$

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

Internal standard solution—A solution of *n*-octylbenzene in ethanol (99.5) (1 in 300).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 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 (10 μm in particle diameter).

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

Mobile phase: A mixture of methanol and water (20:3).

Flow rate: Adjust so that the retention time of epitiostanol 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, epitiostanol 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 ratios of the peak area of epitiostanol 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.

Mepivacaine Hydrochloride

メピバカイン塩酸塩
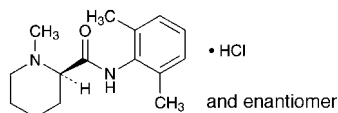
$C_{15}H_{22}N_2O.HCl$: 282.81
(2*RS*)-*N*-(2,6-Dimethylphenyl)-1-methylpiperidine-2-carboxamide monohydrochloride
[*1722-62-9*]

Mepivacaine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$).

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

It is freely soluble in water and in methanol, soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Mepivacaine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 256°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Mepivacaine Hydrochloride (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 Mepivacaine 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 Mepivacaine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.2 g of Mepivacaine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

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

(2) Sulfate <1.14>—Perform the test with 0.5 g of Mepivacaine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Mepivacaine 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).

(4) Related substances—Dissolve 0.10 g of Mepivacaine Hydrochloride in 5 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 2 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 for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol and ammonia solution (28) (100:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly bismuth nitrate-potassium iodide 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% (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 Mepivacaine Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100) and add 70 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
= 28.28 mg of $C_{15}H_{22}N_2O.HCl$

Containers and storage Containers—Tight containers.

Mepivacaine Hydrochloride Injection

メピバカイン塩酸塩注射液

Mepivacaine Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$: 282.81).

Method of preparation Prepare as directed under Injections, with Mepivacaine Hydrochloride.

Description Mepivacaine Hydrochloride Injection is a clear, colorless liquid.

Identification To a volume of Mepivacaine Hydrochloride Injection, equivalent to 20 mg of Mepivacaine Hydrochlo-

ride, add 1 mL of sodium hydrochloride TS, and extract with 20 mL of hexane. To 8 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, shake vigorously, and determine the absorption spectrum of the water layer separated as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 261 nm and 265 nm, and between 270 nm and 273 nm.

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

Bacterial endotoxins <4.01>   Less than 0.6 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 Mepivacaine Hydrochloride Injection, equivalent to about 40 mg of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$), add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of mepivacaine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloride TS 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 mepivacaine to that of the internal standard.

$$\text{Amount (mg) of mepivacaine hydrochloride}$$
$$(C_{15}H_{22}N_2O.HCl)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of mepivacaine hydrochloride for assay taken

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column about 4 mm in inside diameter and 15 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: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution (pH 3.0) and acetonitrile (11:9).
Flow rate: Adjust so that the retention time of mepivacaine 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, mepivacaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.
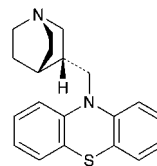System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the

peak area of mepivacaine to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Hermetic containers.

Mequitazine

メキタジン

and enantiomer

$C_{20}H_{22}N_2S$: 322.47
10-[(3*RS*)-1-Azabicyclo[2.2.2]oct-3-ylmethyl]-10*H*-phenothiazine
[*29216-28-2*]

Mequitazine contains not less than 98.5% of mequitazine ($C_{20}H_{22}N_2S$), calculated on the dried basis.

Description   Mequitazine occurs as white, crystals or crystalline powder.
It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and practically insoluble in water.
It is gradually colored by light.
A solution of Mequitazine in methanol (1 in 50) shows no optical rotation.

Identification   **(1)**   Determine the absorption spectrum of a solution of Mequitazine in ethanol (95) (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 Mequitazine, 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>   146 – 150°C

Purity   **(1)**   Heavy metals <1.07>—Proceed with 1.0 g of Mequitazine 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 Mequitazine 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 50 mL, then 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 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of ethyl acetate, methanol and diethylamine (7:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 3 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, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

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

Assay Weigh accurately about 0.25 g of Mequitazine, dissolve in 50 mL of acetic acid (100), 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
= 32.25 mg of $C_{20}H_{22}N_2S$

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

Mequitazine Tablets

メキタジン錠

Mequitazine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mequitazine ($C_{20}H_{22}N_2S$: 322.47).

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

Identification Powder Mequitazine Tablets. To a portion of the powder, equivalent to 3 mg of Mequitazine, add 50 mL of ethanol (95), shake thoroughly, and add ethanol (95) to make 100 mL. Centrifuge, if necessary, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 µm. Discard 10 mL of the first filtrate, to 4 mL of the subsequent filtrate add ethanol (95) to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 253 nm and 257 nm and between 301 nm and 311 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 Mequitazine Tablets add 50 mL of a mixture of methanol and water (4:3), and disperse to fine particles with the aid of ultrasonic waves. Shake this solution thoroughly, and add methanol to make exactly 100 mL. Centrifuge, if necessary, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 µm. Discard 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V' mL so that each mL contains about 4.8 µg of mequitazine ($C_{20}H_{22}N_2S$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of mequitazine ($C_{20}H_{22}N_2S$)
= $M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of mequitazine 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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mequitazine Tablets is not less than 70%.

Start the test with 1 tablet of Mequitazine 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 10 mL of the first 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 mequitazine ($C_{20}H_{22}N_2S$), and use this solution as the sample solution. Separately, weigh accurately about 15 mg of mequitazine for assay, previously dried in vacuum at 60°C using phosphorous (V) oxide as the desiccant for 3 hours, dissolve in 50 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 200 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 253 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of mequitazine ($C_{20}H_{22}N_2S$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$

M_S: Amount (mg) of mequitazine for assay taken
C: Labeled amount (mg) of mequitazine ($C_{20}H_{22}N_2S$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Mequitazine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of mequitazine ($C_{20}H_{22}N_2S$), add 50 mL of a mixture of methanol and water (4:3), shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, if necessary, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 µm. Discard 10 mL of the first filtrate, pipet 4 mL of the subsequent filtrate, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of mequitazine for assay, previously dried in vacuum at 60°C using phosphorous (V) oxide as the desiccant for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol 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 254 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of mequitazine ($C_{20}H_{22}N_2S$)
= $M_S \times A_T/A_S \times 1/8$

M_S: Amount (mg) of mequitazine for assay taken

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

Mercaptopurine Hydrate

メルカプトプリン水和物
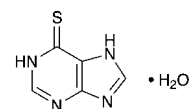
$C_5H_4N_4S \cdot H_2O$: 170.19
1,7-Dihydro-6*H*-purine-6-thione monohydrate
[*6112-76-1*]

Mercaptopurine Hydrate contains not less than 98.0% of mercaptopurine ($C_5H_4N_4S$: 152.18), calculated on the anhydrous basis.

Description Mercaptopurine Hydrate occurs as light yellow to yellow, crystals or crystalline powder. It is odorless.

It is practically insoluble in water, in acetone and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification (1) Dissolve 0.6 g of Mercaptopurine Hydrate in 6 mL of sodium hydroxide solution (3 in 100), and add slowly 0.5 mL of iodomethane with vigorous stirring. Stir well for 10 minutes, cool in an ice bath, and adjust the pH with acetic acid (31) to about 5. Collect the separated crystals by filtration, recrystallize from water, and dry at 120°C for 30 minutes: the crystals melt <2.60> between 218°C and 222°C (with decomposition).

(2) Determine the absorption spectrum of a solution of Mercaptopurine Hydrate 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.

Purity (1) Clarity of solution—Dissolve 0.20 g of Mercaptopurine Hydrate in 10 mL of ammonia TS: the solution is clear.

(2) Sulfate <1.14>—Dissolve 50 mg of Mercaptopurine Hydrate in 10 mL of dilute hydrochloric acid, add 5 drops of barium chloride TS, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Mercaptopurine 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).

(4) Hypoxanthine—Dissolve 50 mg of Mercaptopurine Hydrate in exactly 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the sample solution. Separately, dissolve 5.0 mg of hypoxanthine in a solution of ammonia solution (28) in methanol (1 in 10) 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 methanol, chloroform, *n*-butyl formate and ammonia solution (28) (8:6:4:1) 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 observed at the same place as that from the standard solution, is not larger and not more intense than that from the standard solution.

(5) Phosphorus—Take 0.20 g of Mercaptopurine Hydrate in a crucible, add 2 mL of diluted sulfuric acid (3 in 7), then heat gently, slowly adding dropwise several 0.5-mL portions of nitric acid, until the liquid becomes colorless. Continue to heat until most of the liquid has evaporated, cool, and dissolve the residue in 10 mL of water. Transfer the solution to a 25-mL volumetric flask, wash the crucible with two 4-mL portions of water, combine the washings with the solution in the volumetric flask, and use this solution as the sample solution. Separately, dissolve 0.4396 g of potassium dihydrogen phosphate in water to make exactly 200 mL. To 2.0 mL of this solution add water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 16 mL of water, and use this solution as the standard solution. To the sample solution and standard solution add 1 mL of diluted sulfuric acid (3 in 7), 0.5 mL of nitric acid, 0.75 mL of hexaammonium heptamolybdate TS, 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS and water to make 25 mL, and allow to stand for 5 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the ab-

sorbance of the subsequent solution of the sample solution at 750 nm is not larger than that of the subsequent solution of the standard solution.

Water <2.48> 10.0 – 12.0% (0.2 g, volumetric titration, back titration).

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

Assay Weigh accurately about 0.25 g of Mercaptopurine Hydrate, dissolve in 90 mL of *N*,*N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination with a mixture of 90 mL of *N*,*N*-dimethylformamide and 15 mL of water, and make any necessary correction.

> Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
> = 15.22 mg of $C_5H_4N_4S$

Containers and storage Containers—Well-closed containers.

Mercurochrome

Merbromin

マーキュロクロム

Mercurochrome is a sodium salt of a mixture of brominated and mercurized fluoresceins.

When dried, it contains not less than 18.0% and not more than 22.4% of bromine (Br: 79.90), and not less than 22.4% and not more than 26.7% of mercury (Hg: 200.59).

Description Mercurochrome occurs as blue-green to greenish red-brown, scales or granules. It is odorless.

It is freely soluble in water, but sometimes leaves a small amount of insoluble matter. It is practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) A solution of Mercurochrome (1 in 2000) shows a red color and a yellow-green fluorescence.

(2) To 5 mL of a solution of Mercurochrome (1 in 250) add 3 drops of dilute sulfuric acid: a reddish orange precipitate is produced.

(3) Heat 0.1 g of Mercurochrome with small crystals of iodine in a test tube: red crystals are sublimed on the upper part of the tube. If yellow crystals are produced, scratch with a glass rod: the color of the crystals changes to red.

(4) Place 0.1 g of Mercurochrome in a porcelain crucible, add 1 mL of a solution of sodium hydroxide (1 in 6), evaporate to dryness with stirring, and ignite. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and shake with 3 drops of chlorine TS and 2 mL of chloroform: a yellowish brown color develops in the chloroform layer.

Purity (1) Dyestuff—Dissolve 0.40 g of Mercurochrome in 20 mL of water, add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

(2) Soluble halides—Dissolve 5.0 g of Mercurochrome in 80 mL of water, add 10 mL of dilute nitric acid and water to make 100 mL, shake, and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes protected from direct sunlight: no turbidity is produced, or even if produced, it is not more than that of the following control solu-

tion.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, and proceed as directed above.

(3) Soluble mercury salts—To 5 mL of the filtrate obtained in (1) add 5 mL of water, and use this solution as the sample solution. Dissolve 40 mg of mercury (II) chloride, weighed accurately, in water to make 1000 mL, and add 3 mL of dilute sulfuric acid to 20 mL of this solution. To 5 mL of the solution add 5 mL of water, and use this as the control solution. Add 1 drop each of sodium sulfide TS to these solutions, and compare: the sample solution has no more color than the control solution.

(4) Insoluble mercury compounds—Dissolve 2.5 g of Mercurochrome in 50 mL of water, allow to stand for 24 hours, centrifuge, and wash the precipitate with small portions of water until the last washing becomes colorless. Transfer the precipitate to a glass-stoppered flask, add exactly 5 mL of 0.05 mol/L iodine VS, allow to stand for 1 hour with frequent agitation, add 4.3 mL of 0.1 mol/L sodium thiosulfate VS dropwise with shaking, and add 1 mL of starch TS: a blue color develops.

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 5 hours).

Assay (1) Mercury—Weigh accurately about 0.6 g of Mercurochrome, previously powdered and dried, transfer to an iodine flask, dissolve in 50 mL of water, add 8 mL of acetic acid (31), 20 mL of chloroform and exactly 30 mL of 0.05 mol/L iodine VS, stopper tightly, and allow to stand for 1 hour with frequent, vigorous shaking. Titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS with vigorous shaking (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

(2) Bromine—Weigh accurately about 0.5 g of Mercurochrome, previously powdered and dried, in a porcelain crucible, add 2 g of potassium nitrate, 3 g of potassium carbonate and 3 g of anhydrous sodium carbonate, mix well, cover the surface of the mixture with 3 g of a mixture of equal amounts of potassium carbonate and anhydrous sodium carbonate, and ignite almost to fusion. Cool, dissolve the ignited mixture in 80 mL of warm water, acidify with nitric acid, and add exactly 25 mL of 0.1 mol/L silver nitrate VS. Shake well, 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 and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 7.990 mg of Br

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

Mercurochrome Solution

Merbromin Solution

マーキュロクロム液

Mercurochrome Solution contains not less than 0.42 w/v% and not more than 0.56 w/v% of mercury (Hg: 200.59).

Method of preparation

Mercurochrome	20 g
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare by mixing the above ingredients.

Description Mercurochrome Solution is a dark red liquid.

Identification (1) To 1 mL of Mercurochrome Solution add 40 mL of water: the resulting solution shows a red color and a yellow-green fluorescence.

(2) Dilute 1 mL of Mercurochrome Solution with 4 mL of water, and add 3 drops of dilute sulfuric acid: a red-orange precipitate is produced.

(3) Evaporate 5 mL of Mercurochrome Solution to dryness, and proceed with the residue as directed in the Identification (3) under Mercurochrome.

(4) To 5 mL of Mercurochrome Solution add 1 mL of a solution of sodium hydroxide (1 in 6), and proceed as directed in the Identification (4) under Mercurochrome.

Purity Dyestuff—To 20 mL of Mercurochrome Solution add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

Assay Transfer exactly measured 30 mL of Mercurochrome Solution to an iodine flask, dilute with 20 mL of water, add 8 mL of acetic acid (31) and 20 mL of chloroform, and proceed as directed in the Assay (1) under Mercurochrome.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

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

Meropenem Hydrate

メロペネム水和物
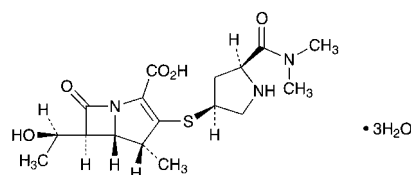
$C_{17}H_{25}N_3O_5S.3H_2O$: 437.51
(4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-(Dimethylcarbamoyl)pyrrolidin-3-ylsulfanyl]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate
[*119478-56-7*]

Meropenem 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 Meropenem Hydrate is expressed as mass (potency) of meropenem ($C_{17}H_{25}N_3O_5S$: 383.46).

Description   Meropenem Hydrate occurs as a white to light yellow crystalline powder.

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

It dissolves in sodium hydrogen carbonate TS.

Identification   (1)   Dissolve 10 mg of Meropenem Hydrate in 2 mL of water, add 3 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2)   Determine the absorption spectra of solutions of Meropenem Hydrate and Meropenem RS (3 in 100,000) 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 Meropenem Hydrate and Meropenem 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> $[\alpha]_D^{20}$: $-17 - -21°$ (0.22 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

pH <2.54>   Dissolve 0.2 g of Meropenem 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 Meropenem Hydrate in 10 mL of sodium hydrogen carbonate TS: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 18.5 mL of diluted hydrochloric acid (1 in 40).

(2)   Heavy metals <1.07>—Proceed with 2.0 g of Meropenem 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).

(3)   Related substances—Dissolve 50 mg of Meropenem Hydrate in 10 mL of triethylamine-phosphate buffer solution (pH 5.0), and use this solution as the sample solution. Prepare the sample solution before use. Pipet 1 mL of the sample solution, and add triethylamine-phosphate buffer solution (pH 5.0) to make exactly 100 mL. Pipet 3 mL of this solution, add triethylamine-phosphate buffer solution (pH 5.0) 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 ring-opened meropenem, having the relative retention time about 0.5 to meropenem, and the peak area of the dimmer, having the relative retention time about 2.2 to meropenem, obtained from the sample solution are not larger than the peak area of meropenem obtained from the standard solution, the area of the peak other than meropenem and the peaks mentioned above from the sample solution is not larger than 1/3 times the peak area of meropenem from the standard solution, and the total area of the peaks other than meropenem from the sample solution is not larger than 3 times the peak area of meropenem from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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: A mixture of triethylamine-phosphate buffer solution (pH 5.0) and acetonitrile (100:7).

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

Time span of measurement: About 7 times as long as the retention time of meropenem.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add triethylamine-phosphate buffer solution (pH 5.0) to make exactly 25 mL. Confirm that the peak area of meropenem obtained from 10 μL of this solution is equivalent to 16 to 24% of that obtained from 10 μL of the standard solution.

System performance: Warm the sample solution at 60°C for 30 minutes. When the procedure is run with 10 μL of this solution under the above operating conditions, the ring-opened meropenem, meropenem and the dimer are eluted in this order, and the resolution between the peaks of the ring-opened meropenem and meropenem is 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 meropenem is not more than 1.5%.

Water <2.48>   Not less than 11.4% and not more than 13.4% (0.35 g, volumetric titration, direct titration).

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

Assay   Weigh accurately an amount of Meropenem Hydrate and Meropenem RS, equivalent to about 50 mg (potency), dissolve each in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution (pH 5.0) to make 100 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 meropenem to that of the internal standard.

Amount [μg (potency)] of meropenem ($C_{17}H_{25}N_3O_5S$)
    $= M_S \times Q_T/Q_S \times 1000$

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

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution (pH 5.0) (1 in 300).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 25°C.

Mobile phase: A mixture of triethylamine-phosphate buffer solution (pH 5.0) and methanol (5:1).

Flow rate: Adjust so that the retention time of meropenem 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, meropenem 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 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 meropenem to that of the internal standard is not more than 1.0%.

Containers and storage　Containers—Tight containers.

Meropenem for Injection

注射用メロペネム

Meropenem for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of meropenem (C$_{17}$H$_{25}$N$_3$O$_5$S: 383.46).

Method of preparation　Prepare as directed under Injections, with Meropenem Hydrate.

Description　Meropenem for Injection occurs as a white to light yellow crystalline powder.

Identification　Determine the infrared absorption spectrum of Meropenem for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3410 cm^{-1}, 1750 cm^{-1}, 1655 cm^{-1}, 1583 cm^{-1} and 1391 cm^{-1}.

pH <2.54>　Dissolve an amount of Meropenem for Injection, equivalent to 0.25 g (potency) of Meropenem Hydrate, in 5 mL of water: the pH of the solution is between 7.3 and 8.3.

Purity　(1) Clarity and color of solution—Dissolve an amount of Meropenem for Injection, equivalent to 1.0 g (potency) of Meropenem Hydrate, in 20 mL of water: the solution is clear and is not more intensely colored than the following control solution.

Control solution: To a mixture of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 18.5 mL of diluted hydrochloric acid (1 in 40).

(2) Related substances—Dissolve an amount of Meropenem for Injection, equivalent to 0.10 g (potency) of Meropenem Hydrate, in triethylamine-phosphate buffer solution (pH 5.0) to make 25 mL, and use this solution as the sample solution. Prepare the sample solution before use. Pipet 1 mL of the sample solution, add triethylamine-phosphate buffer solution (pH 5.0) to make exactly 100 mL. Pipet 5 mL of this solution, add triethylamine-phosphate buffer solution (pH 5.0) 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 peak area of ring-opened meropenem and meropenem dimer, respectively having the relative retention time of about 0.5 and about 2.2 to meropenem obtained from the sample solution is not larger than the peak area of meropenem obtained from the standard solution, the area of the peak, other than meropenem and the peaks mentioned above, is not larger than 1/5

times the peak area of meropenem obtained from the standard solution, and the total area of the peaks other than meropenem is not larger than 3 times the peak area of meropenem obtained from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Meropenem Hydrate.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add triethylamine-phosphate buffer solution (pH 5.0) to make exactly 25 mL. Confirm that the peak area of meropenem obtained with 10 μL of this solution is equivalent to 16 to 24% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the sample solution, previously allowed to stand at 60°C for 30 minutes, under the above operating conditions, the ring-opened meropenem, meropenem and the meropenem dimer are eluted in this order, and the resolution between the peaks of the ring-opened meropenem and meropenem is 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 meropenem is not more than 1.5%.

Loss on drying <2.41>　9.5 – 12.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <4.01>　Less than 0.12 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 Meropenem for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Meropenem Hydrate, dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution (pH 5.0) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Meropenem RS, equivalent to about 50 mg (potency), dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution (pH 5.0) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Meropenem Hydrate.

Amount [mg (potency)] of meropenem (C$_{17}$H$_{25}$N$_3$O$_5$S)
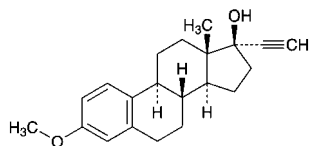　= $M_S \times Q_T/Q_S$

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

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution (pH 5.0) (1 in 300).

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

Mestranol

メストラノール

C$_{21}$H$_{26}$O$_2$: 310.43
3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol
[*72-33-3*]

Mestranol, when dried, contains not less than 97.0% and not more than 102.0% of mestranol (C$_{21}$H$_{26}$O$_2$).

Description Mestranol occurs as a white to pale yellowish white crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification **(1)** Dissolve 2 mg of Mestranol in 1 mL of a mixture of sulfuric acid and ethanol (99.5) (2:1): a red-purple color develops with a yellow-green fluorescence.

(2) Determine the absorption spectrum of a solution of Mestranol in ethanol (99.5) (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 Mestranol 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 Mestranol, 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 Mestranol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]$_D^{20}$: +2 – +8° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 148 – 154°C

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Mestranol 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 Mestranol according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Mestranol in 20 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 for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) 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 10 mg each of Mestranol and Mestranol RS, previously dried, dissolve in ethanol (99.5) 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 the standard solution at 279 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

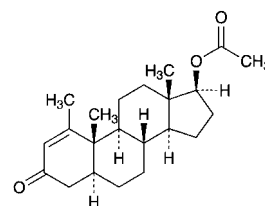Amount (mg) of mestranol (C$_{21}$H$_{26}$O$_2$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Mestranol RS taken

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

Metenolone Acetate

メテノロン酢酸エステル

C$_{22}$H$_{32}$O$_3$: 344.49
1-Methyl-3-oxo-5α-androst-1-en-17β-yl acetate
[*434-05-9*]

Metenolone Acetate, when dried, contains not less than 97.0% and not more than 103.0% of metenolone acetate (C$_{22}$H$_{32}$O$_3$).

Description Metenolone Acetate occurs as a white to pale yellowish white crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in methanol and in ethanol (95), sparingly soluble in diethyl ether and in sesame oil, slightly soluble in hexane and in petroleum ether, and practically insoluble in water.

Identification **(1)** Dissolve 1 mg of Metenolone Acetate in 5 mL of a mixture of sulfuric acid and ethanol (95) (1:1), and heat for 30 minutes in a water bath: a red-brown color develops.

(2) To 10 mg of Metenolone Acetate add 0.5 mL of dilute sodium hydroxide-ethanol TS, and heat for 1 minute on a water bath. After cooling, add 0.5 mL of diluted sulfuric acid (1 in 2), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(3) Dissolve 50 mg of Metenolone Acetate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), and boil for 2 hours under a reflux condenser. After cooling, add this solution gradually to 50 mL of cold water, and stir for 15 minutes. Filter the precipitate so obtained by suction through a glass filter (G4), wash with 10 mL of water, and dry at 105°C for 1 hour: it melts <2.60> between 157°C and 161°C.

(4) Determine the infrared absorption spectrum of Metenolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Refer-

ence Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+39 - +42°$ (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 141 – 144°C

Purity **(1)** Clarity and color of solution—Dissolve 0.50 g of Metenolone Acetate in 10 mL of 1,4-dioxane: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metenolone 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).

(3) Related substances—Dissolve 35 mg of Metenolone Acetate in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, dilute with chloroform to 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 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 cyclohexane (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% (0.5 g, 105°C, 3 hours).

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

Assay Weigh accurately about 10 mg of Metenolone Acetate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of metenolone acetate $(C_{22}H_{32}O_3)$
= $A/391 \times 10,000$

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

Metenolone Enanthate

メテノロンエナント酸エステル
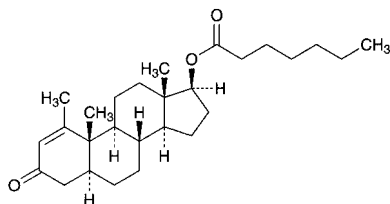
$C_{27}H_{42}O_3$: 414.62
1-Methyl-3-oxo-5α-androst-1-en-17β-yl heptanoate
[*303-42-4*]

Metenolone Enanthate, when dried, contains not less than 97.0% and not more than 103.0% of metenolone enanthate $(C_{27}H_{42}O_3)$.

Description Metenolone Enanthate occurs as white, crystals or crystalline powder. It is odorless.

It is very soluble in ethanol (95), in acetone, in 1,4-dioxane and in chloroform, freely soluble in methanol, in ethyl acetate, in diethyl ether, in cyclohexane, in petroleum ether and in toluene, soluble in sesame oil, and practically insoluble in water.

Identification **(1)** Heat 1 mg of Metenolone Enanthate with 5 mL of a mixture of sulfuric acid and ethanol (95) (1:1) on a water bath for 30 minutes: a red-brown color develops.

(2) Dissolve 50 mg of Metenolone Enanthate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), boil under a reflux condenser for 2 hours, cool, add slowly this solution to 50 mL of cold water, and stir for 15 minutes. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the washings become neutral, and dry at 105°C for 1 hour: it melts <2.60> between 156°C and 162°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+39 - +43°$ (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 67 – 72°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Metenolone Enanthate in 10 mL of 1,4-dioxane: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metenolone Enanthate 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 20 mg of Metenolone Enanthate in exactly 10 mL of chloroform, 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 ethyl acetate and cyclohexane (1:1) 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 does not appear.

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 0.1 g of Metenolone Enanthate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute with methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute again with methanol to make exactly 100 mL. Determine the absorbance, A, of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of metenolone enanthate $(C_{27}H_{42}O_3)$
= $A/325 \times 100,000$

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

Metenolone Enanthate Injection

メテノロンエナント酸エステル注射液

Metenolone Enanthate Injection is an oily solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of metenolone enanthate ($C_{27}H_{42}O_3$: 414.62).

Method of preparation　Prepare as directed under Injections, with Metenolone Enanthate.

Description　Metenolone Enanthate Injection is a clear, pale yellow, oily liquid.

Identification　**(1)**　Measure a volume of Metenolone Enanthate Injection, equivalent to 0.1 g of Metenolone Enanthate, add 20 mL of petroleum ether, and extract with three 20-mL portions of diluted acetic acid (100) (5 in 7). Combine the extracts, wash with 20 mL of petroleum ether, add 300 mL of cold water while cooling in an ice bath, and stir sufficiently. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the last washing becomes neutral, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 6 hours. With this sample, proceed as directed in the Identification (1) under Metenolone Enanthate.

(2)　Measure a volume of Metenolone Enanthate Injection, equivalent to 10 mg of Metenolone Enanthate, dissolve in 10 mL of chloroform, and use this solution as the sample solution. Separately dissolve 10 mg of metenolone enanthate in 10 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 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 toluene to a distance of about 15 cm, and air-dry the plate. Again develop this plate with a mixture of ethyl acetate and cyclohexane (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same Rf 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.

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 Metenolone Enanthate Injection, equivalent to about 0.1 g of metenolone enanthate ($C_{27}H_{42}O_3$), add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 0.1 g of metenolone enanthate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and prepare the standard solution in the same manner as directed for the preparation of the sample solution. Pipet 3 mL each of the sample solution and standard solution, add exactly 10 mL of isoniazid TS, add methanol to make exactly 20 mL, and allow to stand for 60 minutes. Determine the absorbances, A_T and A_S, of

the solutions from the sample solution and standard solution, respectively, at 384 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained by proceeding with 3 mL of chloroform as the blank.
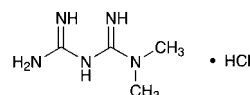
Amount (mg) of metenolone enanthate ($C_{27}H_{42}O_3$)
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of metenolone enanthate for assay taken

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

Metformin Hydrochloride

メトホルミン塩酸塩

$C_4H_{11}N_5.HCl$: 165.62
1,1-Dimethylbiguanide monohydrochloride
[*1115-70-4*]

Metformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of metformin hydrochloride ($C_4H_{11}N_5.HCl$).

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

It is freely soluble in water, sparingly soluble in acetic acid (100), and slightly soluble in ethanol (99.5).

Melting point: about 221°C (with decomposition).

Identification　**(1)**　Determine the absorption spectrum of a solution of Metformin 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 Metformin 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 Metformin Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity　**(1)**　Heavy metals <1.07>—Proceed with 2.0 g of Metformin 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).

(2)　Related substances—Dissolve 2.5 g of Metformin Hydrochloride in 10 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 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add water to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to 0.10 g of 1-cyanoguanidine add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution (3). 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), (2) and (3) on a plate of cellulose

for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, 2-methoxyethanol, water and acetic acid (100) (30:20:5:3) to a distance of about 10 cm, air-dry the plate, then dry at 105°C for 10 minutes. Spray evenly sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS on the plate: the spot other than the principal spot with the sample solution is not more intense than the spot with the standard solution (1), the number of them showing more intense than the spot with the standard solution (2) is not more than two, and the spot with the sample solution appeared at the position corresponding to the spot with the standard solution (3) is not more intense than the spot with the standard solution (3).

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 0.1 g of Metformin Hydrochloride, previously dried, dissolve in 40 mL of acetic acid (100), add 40 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.

$$\text{Each mL of 0.05 mol/L perchloric acid VS} = 4.141 \text{ mg of } C_4H_{11}N_5.HCl$$

Containers and storage   Containers—Tight containers.

Metformin Hydrochloride Tablets

メトホルミン塩酸塩錠

Metformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5.HCl$: 165.62).

Method of preparation   Prepare as directed under Tablets, with Metformin Hydrochloride.

Identification   Shake an amount of powdered Metformin Hydrochloride Tablets, equivalent to 250 mg of Metformin Hydrochloride, with 25 mL of 2-propanol, and filter. Evaporate the filtrate under reduced pressure in a water bath at 40°C, and determine the infrared absorption spectrum of the residue as directed in the potassium chloride disk method under Infrared Spectrophotometry *<2.25>*: it exhibits absorption at the wave numbers of about 3370 cm^{-1}, 3160 cm^{-1}, 1627 cm^{-1}, 1569 cm^{-1} and 1419 cm^{-1}.

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 Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of metformin hydrochloride ($C_4H_{11}N_5.HCl$), add 70 mL of a mixture of water and acetonitrile (3:2), shake for 10 minutes, add the mixture of water and acetonitrile (3:2) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 3 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) to make 50 mL, and use this so-

lution as the sample solution. Separately, weigh accurately about 0.15 g of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the mixture of water and acetonitrile (3:2) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) 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 metformin to that of the internal standard.

$$\text{Amount (mg) of metformin hydrochloride } (C_4H_{11}N_5.HCl) = M_S \times Q_T/Q_S$$

   M_S: Amount (mg) of metformin hydrochloride for assay taken

Internal standard solution—Dissolve 0.3 g of isobutyl parahydroxybenzoate in 100 mL of the mixture of water and acetonitrile (3:2).
Operating conditions—
   Detector: An ultraviolet absorption photometer (wavelength: 235 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 0.8 g of sodium lauryl sulfate in 620 mL of diluted phosphoric acid (1 in 2500), and add 380 mL of acetonitrile.
   Flow rate: Adjust so that the retention time of metformin 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, metformin 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metformin to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Methamphetamine Hydrochloride

メタンフェタミン塩酸塩
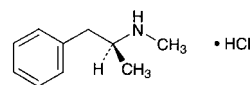
$C_{10}H_{15}N.HCl$: 185.69
(2*S*)-*N*-Methyl-1-phenylpropan-2-amine monohydrochloride
[*51-57-0*]

Methamphetamine Hydrochloride, when dried, contains not less than 98.5% of methamphetamine hydrochloride ($C_{10}H_{15}N.HCl$).

Description   Methamphetamine Hydrochloride occurs as colorless crystals or a white crystalline powder. It is odorless.

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

The pH of a solution of 1.0 g of Methamphetamine Hydrochloride in 10 mL of water is between 5.0 and 6.0.

Identification **(1)** To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of hydrogen hexachloroplatinate (IV) TS: an orange-yellow, crystalline precipitate is produced.

(2) To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of iodine TS: a brown precipitate is produced.

(3) To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of 2,4,6-trinitrophenol TS: a yellow, crystalline precipitate is produced.

(4) A solution of Methamphetamine Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +16 – +19° (after drying, 0.2 g, water, 10 mL, 100 mm).

Melting point <*2.60*> 171 – 175°C

Purity **(1)** Acidity or alkalinity—Dissolve 2.0 g of Methamphetamine Hydrochloride in 40 mL of freshly boiled and cooled water, add 2 drops of methyl red TS, and use this solution as the sample solution.

(i) To 20 mL of the sample solution add 0.20 mL of 0.01 mol/L sulfuric acid VS: a red color develops.

(ii) To 20 mL of the sample solution add 0.20 mL of 0.02 mol/L sodium hydroxide VS: a yellow color develops.

(2) Sulfate <*1.14*>—Dissolve 0.05 g of Methamphetamine 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: the solution remains unchanged.

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 0.4 g of Methamphetamine Hydrochloride, previously dried, and 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.
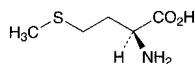
Each mL of 0.1 mol/L perchloric acid VS
= 18.57 mg of $C_{10}H_{15}N.HCl$

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

L-Methionine

L-メチオニン

H_3C-S CO_2H

H NH_2

$C_5H_{11}NO_2S$: 149.21
(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid
[*63-68-3*]

L-Methionine, when dried, contains not less than 98.5% of L-methionine ($C_5H_{11}NO_2S$).

Description L-Methionine occurs as white, crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in formic acid, soluble in water, and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Methionine, 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*> $[\alpha]_D^{20}$: +21.0 – +25.0° (after drying, 0.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.5 g of L-Methionine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Methionine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.5 g of L-Methionine in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 40 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, 6 mL of dilute nitric acid and water to make 40 mL. In this test, to the test solution and the control solution add 10 mL each of silver nitrate TS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Methionine. 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-Methionine. 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-Methionine in 40 mL of water and 2 mL of dilute acetic acid, dissolve 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*>—Transfer 1.0 g of L-Methionine to a 100-mL decomposition 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 two 2-mL portions of nitric acid, heat, add 2-mL portions of hydrogen peroxide (30) several times, and heat until the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Methionine in 10 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. After air-drying, immediately 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) on the plate, 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 0.15 g of L-Methionine, 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.

<div align="center">
Each mL of 0.1 mol/L perchloric acid VS
= 14.92 mg of $C_5H_{11}NO_2S$
</div>

Containers and storage Containers—Tight containers.

Methotrexate

メトトレキサート
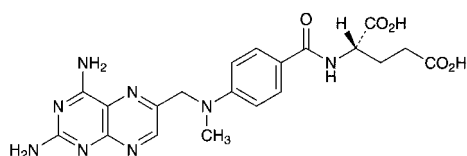
$C_{20}H_{22}N_8O_5$: 454.44
N-{4-[(2,4-Diaminopteridin-
6-ylmethyl)(methyl)amino]benzoyl}-L-glutamic acid
[*59-05-2*]

Methotrexate is a mixture of 4-amino-10-methylfolic acid and closely related compounds.

It contains not less than 94.0% and not more than 102.0% of methotrexate ($C_{20}H_{22}N_8O_5$), calculated on the anhydrous basis.

Description Methotrexate occurs as a yellow-brown crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetonitrile, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

Identification **(1)** Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. 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 Methotrexate 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 Methotrexate 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 Methotrexate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water <*2.48*> Take 5 mL of pyridine for water determination and 20 mL of methanol for water determination in a dried titration flask, and titrate with Karl Fischer TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS for water determination. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

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

Assay Weigh accurately about 25 mg each of Methotrexate and Methotrexate RS, dissolve in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each 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 methotrexate in each solution.

<div align="center">
Amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$) = $M_S \times A_T/A_S$
</div>

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 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 (10 μm in particle diameter).

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

Mobile phase: A mixture of disodium hydrogen phosphate-citric acid buffer solution (pH 6.0) and acetonitrile (89:11).

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

System suitability—

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, folic acid and methotrexate 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 peak area of methotrexate is not more than 1.0%.

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

Methotrexate Capsules

メトトレキサートカプセル

Methotrexate Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$: 454.44).

Method of preparation Prepare as directed under Capsules, with Methotrexate.

Identification To an amount of the content of Methotrexate Capsules, equivalent to 2 mg of Methotrexate, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 10 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 240 nm and 244 nm and between 304 nm and 308 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 the content of 1 capsule of Methotrexate Capsules add $3V/5$ mL of the mobile phase, agitate with the aid of ultrasonic waves for 15 minutes, then shake for 25 minutes, and add the mobile phase to make exactly V mL so that each mL

contains about 20 μg of methotrexate ($C_{20}H_{22}N_8O_5$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water <2.48> in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase 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 methotrexate to that of the internal standard.

Amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$)
= $M_S \times Q_T/Q_S \times V/500$

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

Internal standard solution—A solution of 4-nitrophenol in methanol (1 in 10,000).
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 ratio of the peak area of methotrexate to that of the internal standard 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 the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Methotrexate Capsules is not less than 85%.

Start the test with 1 capsule of Methotrexate 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 2.2 μg of methotrexate ($C_{20}H_{22}N_8O_5$), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water <2.48> in the same manner as Methotrexate), and dissolve in the mobile phase 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 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 methotrexate in each solution.

Dissolution rate (%) with respect to the labeled amount
of methotrexate ($C_{20}H_{22}N_8O_5$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Methotrexate RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$) 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 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of methotrexate 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 methotrexate is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Methotrexate Capsules, take out all of the content, and accurately weigh the mass of the empty capsules. Powder the content, weigh accurately a portion of the powder, equivalent to about 10 mg of methotrexate ($C_{20}H_{22}N_8O_5$), add 60 mL of the mobile phase, shake for 25 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methotrexate RS (separately determine the water <2.48> in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase 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>, and calculate the ratios, Q_T and Q_S, of the peak area of methotrexate to that of the internal standard.

Amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$)
= $M_S \times Q_T/Q_S$

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

Internal standard solution—A solution of 4-nitrophenol in methanol (1 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 302 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: To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 28.5 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. To 890 mL of this solution add 110 mL of acetonitrile.
Flow rate: Adjust so that the retention time of methotrexate is about 6 minutes.
System suitability—
System performance: Dissolve 10 mg each of methotrexate and folic acid in 100 mL of the mobile phase. To 2 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, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.
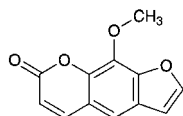System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of

the peak area of methotrexate to that of the internal standard is not more than 1.0%.

Containers and storage　Containers—Tight containers.

Methoxsalen

メトキサレン

$C_{12}H_8O_4$: 216.19
9-Methoxy-7*H*-furo[3,2-*g*]chromen-7-one
[*298-81-7*]

Methoxsalen contains not less than 98.0% and not more than 102.0% of methoxsalen ($C_{12}H_8O_4$), calculated on the anhydrous basis.

Description　Methoxsalen occurs as white to pale yellow, crystals or crystalline powder. It is odorless and tasteless.

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

Identification　(1)　To 10 mg of Methoxsalen add 5 mL of dilute nitric acid, and heat: a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

(2)　To 10 mg of Methoxsalen add 5 mL of sulfuric acid, and shake: a yellow color develops.

(3)　Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (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 Methoxsalen RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60>　145 – 149°C

Purity　(1)　Heavy metals <1.07>—Proceed with 1.0 g of Methoxsalen 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 Methoxsalen according to Method 3, and perform the test (not more than 2 ppm).

(3)　Related substances—Dissolve 50 mg of Methoxsalen in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 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 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, hexane and ethyl acetate (40:10:3) 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.

Water <2.48>　Not more than 0.5% (1 g, volumetric titration, direct titration).

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

Assay　Weigh accurately about 50 mg each of Methoxsalen and Methoxsalen RS, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with 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 the standard solution at 300 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

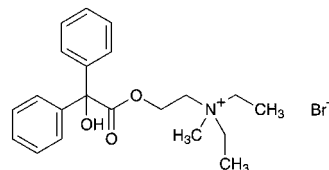Amount (mg) of methoxsalen ($C_{12}H_8O_4$) = $M_S \times A_T/A_S$

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

Containers and storage　Containers—Well-closed containers.
Storage—Light-resistant.

Methylbenactyzium Bromide

メチルベナクチジウム臭化物

$C_{21}H_{28}BrNO_3$: 422.36
N,N-Diethyl-2-[(hydroxyl)(diphenyl)acetoxy]-*N*-methylethylaminium bromide
[*3166-62-9*]

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of methylbenactyzium bromide ($C_{21}H_{28}BrNO_3$).

Description　Methylbenactyzium Bromide occurs as white, crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

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

The pH of a solution of 1.0 g of Methylbenactyzium Bromide in 50 mL of water is between 5.0 and 6.0.

Identification　(1)　Shake 0.5 mL of a solution of Methylbenactyzium Bromide (1 in 100) with 5 mL of phosphate buffer solution (pH 7.0), 2 to 3 drops of bromothymol blue TS and 5 mL of chloroform: a yellow color develops in the chloroform layer.

(2)　To about 1 g of Methylbenactyzium Bromide add 5 mL of water and 10 mL of sodium hydroxide TS, allow to stand for 5 minutes, add 5 mL of dilute hydrochloric acid, collect the precipitate, wash well with water, recrystallize from a mixture of water and ethanol (95) (10:3), and dry at 105°C for 1 hour: the crystals melt <2.60> between 145°C and 150°C. Continue the heating up to about 200°C: a red color develops.

(3)　Add 2 mL of dilute nitric acid to 5 mL of a solution of Methylbenactyzium Bromide (1 in 10): the solution responds to the Qualitative Tests <1.09> (1) for bromide.

Melting point <2.60>　168 – 172°C

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Methylbenactyzium Bromide in 10 mL of water: the solution is clear and colorless.

(2)   Sulfate *<1.14>*—Perform the test with 0.5 g of Methylbenactyzium Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3)   Heavy metals *<1.07>*—Proceed with 2.0 g of Methylbenactyzium 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).

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

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

Assay   Weigh accurately about 0.5 g of Methylbenactyzium Bromide, previously dried, and dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (4:1). Titrate *<2.50>* with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 42.24 mg of $C_{21}H_{28}BrNO_3$

</div>

Containers and storage   Containers—Tight containers.

Methylcellulose

メチルセルロース

[*9004-67-5*]

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 (♦ ♦).

Methylcellulose is a methyl ether of cellulose.

It contains not less than 26.0% and not more than 33.0% of methoxy group (-OCH_3: 31.03), calculated on the dried basis.

The viscosity of Methylcellulose is shown in millipascal second (mPa·s).

♦**Description**   Methylcellulose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

Methylcellulose swells, when water is added, and forms a clear or slightly turbid, viscous liquid.♦

Identification   (1)   Disperse evenly 1.0 g of Methylcellulose 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 Methylcellulose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 5°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, and it does not change 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 Methylcellulose having a labeled viscosity of less than 600 mPa·s. Put an exact amount of Methylcellulose, equivalent to 4.000 g 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 5°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 Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put an exact amount of Methylcellulose, equivalent to 10.00 g 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 cylindertype 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 Methylcellulose 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 glass 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 Methylcellulose, 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 in a reaction vial, 2.0 mL of the internal standard solution and 2.0 mL of hydriodic acid, stopper the vial immediately, and weigh accurately. Add 45 μL of iodomethane for assay through the septum using micro-syringe, weigh accurately, 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_T and Q_S, of the peak area of iodomethane to that of the internal standard.

Content (%) of methoxy group (CH_3O)
$$= M_S/M \times Q_T/Q_S \times 21.86$$

M_S: Amount (mg) of iodomethane for assay taken
M: Amount (mg) of Methylcellulose 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.
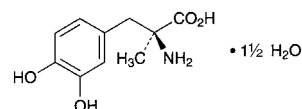
System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane and the internal standard are eluted in this order, with complete separation of these peaks.

◆**Containers and storage**   Containers—Well-closed containers.◆

Methyldopa Hydrate

メチルドパ水和物

$C_{10}H_{13}NO_4.1\frac{1}{2}H_2O$: 238.24
(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate
[*41372-08-1*]

Methyldopa Hydrate contains not less than 98.0% of methyldopa ($C_{10}H_{13}NO_4$: 211.21), calculated on the anhydrous basis.

Description   Methyldopa Hydrate occurs as a white to pale grayish white crystalline powder.

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

It dissolves in dilute hydrochloric acid.

Identification   (1)   To 10 mg of Methyldopa Hydrate add 3 drops of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.

(2)   Determine the absorption spectrum of a solution of Methyldopa Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyldopa 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 Methyldopa 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 Methyldopa RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-25 - -28°$ (1 g calculated on the anhydrous basis, aluminum (III) chloride TS, 20 mL, 100 mm).

Purity **(1)** Acidity—Shake 1.0 g of Methyldopa Hydrate with 100 mL of freshly boiled and cooled water, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color develops.

(2) Chloride <1.03>—Perform the test with 0.5 g of Methyldopa Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Methyldopa 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 Methyldopa Hydrate in 5 mL of dilute hydrochloric acid, and perform the test (not more than 2 ppm).

(5) 3-*O*-Methylmethyldopa—Dissolve 0.10 g of Methyldopa Hydrate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 5 mg of 3-*O*-methylmethyldopa for thin-layer chromatography 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 20 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) (13:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate, and air-dry the plate, then spray evenly a solution of sodium carbonate decahydrate (1 in 4) on the plate: the spot from the sample solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

Water <2.48> 10.0 – 13.0% (0.2 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 Methyldopa Hydrate, dissolve in 80 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 to 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 21.12 mg of $C_{10}H_{13}NO_4$

</div>

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Methyldopa Tablets

メチルドパ錠

Methyldopa Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$: 211.21).

Method of preparation Prepare as directed under Tablets, with Methyldopa Hydrate.

Identification **(1)** To a quantity of powdered Methyldopa Tablets, equivalent to 0.1 g of Methyldopa Hydrate, add 10 mL of water, and heat in a water bath for 5 minutes with occasional shaking. After cooling, centrifuge for 5 minutes at 2000 rotations per minute, apply 1 drop of the supernatant solution to a filter paper, and dry with warm air. Place 1 drop of ninhydrin TS over the spot, and heat for 5 minutes at 100°C: a purple color develops.

(2) To 0.5 mL of the supernatant liquid obtained in (1) add 2 mL of 0.05 mol/L sulfuric acid TS, 2 mL of iron (II) tartrate TS and 4 drops of ammonia TS, and shake well: a deep purple color develops.

(3) To 0.7 mL of the supernatant liquid obtained in (1) add 0.1 mol/L hydrochloric acid TS to make 20 mL. To 10 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 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 Methyldopa Tablets add 50 mL of 0.05 mol/L sulfuric acid TS, shake for 15 minutes, then add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate equivalent to about 5 mg of methyldopa ($C_{10}H_{13}NO_4$), add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution (pH 8.5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (separately determine the loss on drying <2.41> at 125°C for 2 hours), and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution (pH 8.5) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 520 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

<div align="center">

Amount (mg) of methyldopa ($C_{10}H_{13}NO_4$)
= $M_S \times A_T/A_S \times 5/V$

</div>

M_S: Amount (mg) of Methyldopa 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 water as the dissolution medium, the dissolution rate in 60 minutes of Methyldopa Tablets is not less than 75%.

Start the test with 1 tablet of Methyldopa 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 0.8 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 25 μg of methyldopa ($C_{10}H_{13}NO_4$), and use this solution as the sample solution. Separately, weigh accurately about 56 mg of methyldopa for assay (separately determine the loss on drying <2.41> at 125°C for 2 hours), and dissolve in water to make exactly 200 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 the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of methyldopa ($C_{10}H_{13}NO_4$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of methyldopa for assay taken, calculated on the dried basis
C: Labeled amount (mg) of methyldopa ($C_{10}H_{13}NO_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa ($C_{10}H_{13}NO_4$), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa RS (separately determine the loss on drying <2.41> at 125°C for 2 hours), dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution (pH 8.5) to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS 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 520 nm, respectively.
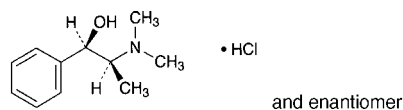
Amount (mg) of methyldopa ($C_{10}H_{13}NO_4$)
$$= M_S \times A_T/A_S$$

M_S: amount (mg) of Methyldopa RS taken, calculated on the dried basis

Containers and storage Containers—Well-closed containers.

dl-**Methylephedrine Hydrochloride**

dl-メチルエフェドリン塩酸塩

• HCl

and enantiomer

$C_{11}H_{17}NO.HCl$: 215.72
(1RS,2SR)-2-Dimethylamino-1-phenylpropan-1-ol monohydrochloride
[*18760-80-0*]

dl-Methylephedrine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of *dl*-methylephedrine hydrochloride

($C_{11}H_{17}NO.HCl$).

Description *dl*-Methylephedrine Hydrochloride occurs as colorless crystals or a white crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (99.5), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride.

A solution of *dl*-Methylephedrine Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of *dl*-Methylephedrine 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 *dl*-Methylephedrine 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 *dl*-Methylephedrine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of *dl*-Methylephedrine Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Melting point <2.60> 207 – 211°C

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

(2) Heavy metals <1.07>—Proceed with 1.0 g of *dl*-Methylephedrine Hydrochloride according to Method 4, 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 50 mg of *dl*-Methylephedrine Hydrochloride in 20 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 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 the peak of methylephedrine from the sample solution is not larger than the peak area of methylephedrine from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 257 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 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.
Flow rate: Adjust so that the retention time of methylephedrine is about 10 minutes.
Time span of measurement: About 2 times as long as the retention time of methylephedrine, 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 methylephedrine 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 50 mg of *dl*-Methylephedrine Hydrochloride and 0.4 mg of methyl parahydroxybenzoate in 50 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, methylephedrine and methyl parahydroxybenzoate 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 peak area of methylephedrine 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 *dl*-Methylephedrine Hydrochloride, 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.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 21.57 mg of $C_{11}H_{17}NO.HCl$

</div>

Containers and storage   Containers—Well-closed containers.

Storage—Light-resistant.

10% *dl*-Methylephedrine Hydrochloride Powder

dl-Methylephedrine Hydrochloride Powder

dl-メチルエフェドリン塩酸塩散 10%

10% *dl*-Methylephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO.HCl$: 215.72).

Method of preparation

dl-Methylephedrine 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% *dl*-Methylephedrine Hydrochloride Powder add 100 mL of water, shake vigorously for 20 minutes, if necessary, filter the solution. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 264 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% *dl*-Methylephedrine Hydrochloride

Powder is not less than 85%.

Start the test with about 0.5 g of 10% *dl*-Methylephedrine 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 2 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add exactly 2 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of *dl*-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 25 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the mobile phase, 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 methylephedrine in each solution.

Dissolution rate (%) with respect to the labeled amount of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO.HCl$)
= $M_S/M_T \times A_T/A_S \times 9/4$

M_S: Amount (mg) of *dl*-methylephedrine hydrochloride for assay taken

M_T: Amount (g) of 10% *dl*-Methylephedrine Hydrochloride Powder taken

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).

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 methylephedrine 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 methylephedrine is not more than 2.0%.

Assay   Weigh accurately about 0.5 g of 10% *dl*-Methylephedrine Hydrochloride Powder, add exactly 4 mL of the internal standard solution and 25 mL of water, shake vigorously for 20 minutes to dissolve, add water to make 50 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, if necessary, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of *dl*-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 4 mL of the internal standard solution and water 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 of the peak area, Q_T and Q_S, of methylephedrine to that of the internal standard.

Amount (mg) of *dl*-methylephedrine hydrochloride
($C_{11}H_{17}NO.HCl$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of *dl*-methylephedrine hydrochloride for assay taken

Internal standard solution—A solution of methyl parahy-

droxybenzoate in acetonitrile (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 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 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust so that the retention time of methylephedrine 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, methylephedrine 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 methylephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methylergometrine Maleate

メチルエルゴメトリンマレイン酸塩
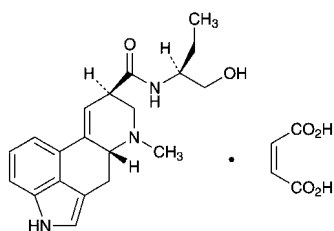
C$_{20}$H$_{25}$N$_3$O$_2$.C$_4$H$_4$O$_4$: 455.50
(8S)-N-[(1S)-1-(Hydroxymethyl)propyl]-6-methyl-9,10-didehydroergoline-8-carboxamide monomaleate
[*7054-07-1*]

Methylergometrine Maleate, when dried, contains not less than 95.0% and not more than 105.0% of methylergometrine maleate (C$_{20}$H$_{25}$N$_3$O$_2$.C$_4$H$_4$O$_4$).

Description Methylergometrine Maleate occurs as a white to pale yellow crystalline powder. It is odorless.

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

It gradually changes to yellow by light.

Melting point: about 190°C (with decomposition).

Identification **(1)** A solution of Methylergometrine Maleate (1 in 200) shows a blue fluorescence.

(2) The colored solution obtained in the Assay develops a deep blue in color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylergometrine Maleate RS prepared in the same manner

as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 5 mL of a solution of Methylergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the test solution fades immediately.

Optical rotation <2.49> [α]$_D^{20}$: +44 – +50° (after drying, 0.1 g, water, 20 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 8 mg of Methylergometrine Maleate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately 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, and immediately develop the plate with a mixture of chloroform, methanol and water (75:25:3) to a distance of about 10 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 2.0% (0.2 g, in vacuum, phosphorus (V) oxide, 4 hours).

Assay Weigh accurately about 10 mg each of Methylergometrine Maleate and Methylergometrine Maleate RS, previously dried, add water to make exactly 250 mL, and use these solutions as the sample solution and the standard solution. Pipet 2 mL each of the sample solution and the standard solution separately into brown glassstoppered test tubes, add exactly 4 mL each of 4-dimethylaminobenzaldehyde-iron (III) chloride TS while ice cooling, warm for 10 minutes at 45°C, and allow to stand for 20 minutes at room temperature. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2.0 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 545 nm, respectively.

Amount (mg) of methylergometrine maleate
(C$_{20}$H$_{25}$N$_3$O$_2$.C$_4$H$_4$O$_4$)
= $M_S \times A_T/A_S$

M_S: Amount (mg) of Methylergometrine Maleate RS taken

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Methylergometrine Maleate Tablets

メチルエルゴメトリンマレイン酸塩錠

Methylergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methylergometrine maleate (C$_{20}$H$_{25}$N$_3$O$_2$.C$_4$H$_4$O$_4$: 455.50).

Method of preparation Prepare as directed under Tablets, with Methylergometrine maleate.

Identification **(1)** The sample solution obtained in the

Assay shows a blue fluorescence.

(2) The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 543 nm and 547 nm and between 620 nm and 630 nm.

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 Methylergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, add 10 mL of water, shake for 10 minutes vigorously, and disintegrate the tablet. Add 3 g of sodium chloride and 2 mL of ammonia solution (28), add exactly 25 mL of chloroform, and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly V mL of a solution containing about $5 \mu g$ of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 1.25 mg of Methylergometrine Maleate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, and add 3 g of sodium chloride and 2 mL of ammonia solution (28). Add exactly 25 mL of chloroform, shake vigorously for 10 minutes, and centrifuge for 5 minutes. Discard the water layer, and use the chloroform layer as the standard solution. Pipet 20 mL each of the sample solution and the standard solution separately into brown glass-stoppered centrifuge tubes, add immediately exactly 10 mL of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake for 5 minutes vigorously. Centrifuge these solutions for 5 minutes, take the water layers, and allow them to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

Amount (mg) of methylergometrine maleate
($C_{20}H_{25}N_3O_2.C_4H_4O_4$)
$$= M_S \times A_T/A_S \times V/250$$

M_S: Amount (mg) of Methylergometrine Maleate RS taken

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Methylergometrine Maleate Tablets is not less than 70%.

Start the test with 1 tablet of Methylergometrine 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 $0.8 \mu m$. Discard the first 10 mL of the filtrate, to exactly V mL of the subsequent filtrate add water to make exactly V' mL so that each mL contains about $0.13 \mu g$ of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Methylergometrine Maleate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) 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, then pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine immedi-

ately the intensities of the fluorescence, F_T and F_S, of the sample solution and standard solution at 338 nm as the excitation wavelength and at 427 nm as the fluorescence wavelength as directed under Fluorometry <2.22>.

Dissolution rate (%) with respect to the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$)
$$= M_S \times F_T/F_S \times V'/V \times 1/C \times 9/20$$

M_S: Amount (mg) of Methylergometrine Maleate RS taken
C: Labeled amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methylergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 mg of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$), transfer to a brown separator, add 15 mL of sodium hydrogen carbonate solution (1 in 20), and extract with four 20-mL portions of chloroform. Filter each portion of the chloroform extracts through a pledget of absorbent cotton, previously moistened with chloroform, into another dried, brown separator, combine all the extracts, and use this extract as the sample solution. Separately, weigh accurately about 10 mg of Methylergometrine Maleate RS, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water, and add water to make exactly 100 mL. Pipet 3 mL of this solution, and transfer to a brown separator, proceed in the same manner as the preparation of the sample solution, and use this extract as the standard solution. To each total volume of the sample solution and the standard solution add exactly 25 mL each of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and after vigorous shaking for 5 minutes, allow to stand for 30 minutes. Draw off the water layer, centrifuge, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 545 nm, respectively.

Amount (mg) of methylergometrine maleate
($C_{20}H_{25}N_3O_2.C_4H_4O_4$)
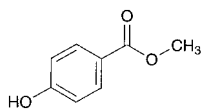$$= M_S \times A_T/A_S \times 3/100$$

M_S: Amount (mg) of Methylergometrine Maleate RS taken

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Methyl Parahydroxybenzoate

パラオキシ安息香酸メチル

C$_8$H$_8$O$_3$: 152.15
Methyl 4-hydroxybenzoate
[*99-76-3*]

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 (◆ ◆).

Methyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of methyl parahydroxybenzoate (C$_8$H$_8$O$_3$).

◆**Description** Methyl Parahydroxybenzoate, occurs as colorless crystals or a white crystalline powder.

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

Identification Determine the infrared absorption spectrum of Methyl 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 Methyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨2.60⟩ 125 – 128°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Methyl 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 Methyl 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 Methyl 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 Methyl 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 con-

ditions, and determine each peak area by the automatic integration method: the peak area of parahydroxybenzoic acid having a relative retention time of about 0.6 to methyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of methyl parahydroxybenzoate obtained from the standard solution (0.5%). For the peak area of parahydroxybenzoic acid, multiply the relative response factor, 1.4. Furthermore, the area of the peak other than methyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of methyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than methyl parahydroxybenzoate from the sample solution is not larger than 2 times the peak area of methyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of methyl 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 5 times as long as the retention time of methyl 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 methyl 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 methyl 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 Methyl Parahydroxybenzoate and Methyl 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 methyl parahydroxybenzoate in each solution.

Amount (mg) of methyl parahydroxybenzoate (C$_8$H$_8$O$_3$)
= $M_S \times A_T/A_S$

M_S: Amount (mg) of Methyl 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 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 and methyl parahydroxybenzoate are eluted in this order, the relative retention time of parahydroxybenzoic acid to methyl parahydroxybenzoate is about 0.6, and the resolution between these peaks 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 methyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage**   Containers—Well-closed containers.◆

Methylprednisolone

メチルプレドニゾロン
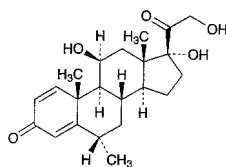
C$_{22}$H$_{30}$O$_5$: 374.47
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione
[*83-43-2*]

Methylprednisolone, when dried, contains not less than 96.0% and not more than 104.0% of methylprednisolone (C$_{22}$H$_{30}$O$_5$).

Description   Methylprednisolone occurs as a white crystalline powder. It is odorless.

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

Melting point: 232 – 240°C (with decomposition).

Identification   (1)   Add 2 mL of sulfuric acid to 2 mg of Methylprednisolone: a deep red color develops with no fluorescence. Then add 10 mL of water to this solution: the color fades, and a gray, flocculent precipitate is produced.

(2)   Dissolve 10 mg of Methylprednisolone in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3)   Determine the absorption spectrum of a solution of Methylprednisolone 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.

Optical rotation <2.49> $[\alpha]_D^{20}$: +79 – +86° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity   Related substances—Dissolve 50 mg of Methylprednisolone 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. 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 dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Then heat at 105°C for 10 minutes, cool, and 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 0.2% (0.2 g).

Assay   Weigh accurately about 10 mg of Methylprednisolone, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of this solution add methanol to make exactly 50 mL, and determine the absorbance A at the wavelength of maximum absorption at about 243 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of methylprednisolone (C}_{22}\text{H}_{30}\text{O}_5\text{)}$$
$$= A/400 \times 10,000$$

Containers and storage   Containers—Tight containers.

Methylprednisolone Succinate

メチルプレドニゾロンコハク酸エステル
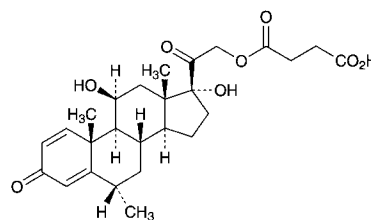
C$_{26}$H$_{34}$O$_8$: 474.54
11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate)
[*2921-57-5*]

Methylprednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of methylprednisolone succinate (C$_{26}$H$_{34}$O$_8$).

Description   Methylprednisolone Succinate occurs as a white, crystals or crystalline powder.

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

Melting point: about 235°C (with decomposition).

It shows crystal polymorphism.

Identification   (1)   Determine the absorption spectrum of a solution of Methylprednisolone Succinate 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 Methylprednisolone Succinate 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 Methylprednisolone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spec-

trophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Methylprednisolone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in ethanol (95), evaporating to dryness, and drying.

Optical rotation <2.49> $[\alpha]_D^{25}$: $+99 \sim +103°$ (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Methylprednisolone Succinate according to Method 4, 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 2.0 g of Methylprednisolone Succinate according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 15 mg of Methylprednisolone Succinate in 5 mL of methanol, add a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (1:1) to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (1:1) 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, and determine each peak area by the automatic integration method: the area of the peaks other than the peak of methylprednisolone succinate from sample solution is not larger than 1/2 times the peak area of methylprednisolone succinate from the standard solution, and the total area of the peaks other than the peak of methylprednisolone succinate is not larger than the peak area of methylprednisolone succinate 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 methylprednisolone succinate.
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 the mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of methylprednisolone succinate obtained from 5 μL of this solution is equivalent to 7 to 13% 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 methylprednisolone succinate is not more than 2.5%.

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).

Assay Weigh accurately about 15 mg each of Methylprednisolone Succinate and Methylprednisolone Succinate RS, previously dried, dissolve separately in 5 mL of methanol, and add the mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, 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 methylprednisolone succinate to that of the internal standard.

Amount (mg) of methylprednisolone succinate ($C_{26}H_{34}O_8$)
= $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Methylprednisolone Succinate RS taken

*Internal standard solution—*A solution of ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (1:1) (3 in 20,000).
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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of 0.05 mol/L disodium hydrogen phosphate TS to make a solution having pH 5.5. To 640 mL of this solution add 360 mL of acetonitrile.

Flow rate: Adjust so that the retention time of methylprednisolone succinate 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, methylprednisolone succinate 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylprednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Methylrosanilinium Chloride

Crystal Violet

メチルロザニリン塩化物

$C_{25}H_{30}ClN_3$: 407.98

Methylrosanilinium Chloride is hexamethylpararosaniline chloride, and is usually admixed with pentamethylpararosaniline chloride and tetramethylpararosaniline chloride.

It contains not less than 96.0% of methylrosanilinium chloride [as hexamethylpararosaniline chloride ($C_{25}H_{30}ClN_3$)], calculated on the dried basis.

Description Methylrosanilinium Chloride occurs as green fragments having a metallic luster or a dark green powder. It is odorless or has a slight odor.

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

Identification **(1)** To 1 mL of sulfuric acid add 1 mg of

Methylrosanilinium Chloride: it dissolves, and shows an orange to red-brown color. To this solution add water dropwise: the color of the solution changes from brown through green to blue.

(2) Dissolve 0.02 g of Methylrosanilinium Chloride in 10 mL of water, add 5 drops of hydrochloric acid, and use this solution as the sample solution. To 5 mL of the sample solution add tannic acid TS dropwise: an intense blue precipitate is formed.

(3) To 5 mL of the sample solution obtained in (2) add 0.5 g of zinc powder, and shake: the solution is decolorized. Place 1 drop of this solution on filter paper, and apply 1 drop of ammonia TS adjacent to it: a blue color is produced at the zone of contact of the both solutions.

Purity **(1)** Ethanol-insoluble substances—Weigh accurately about 1 g of Methylrosanilinium Chloride, previously dried at 105°C for 4 hours, heat with 50 mL of ethanol (95) under a reflux condenser for 15 minutes in a water bath, and filter the mixture through a tared glass filter (G4). Wash the residue on the filter with warm ethanol (95) until the last washing does not show a purple color, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0%.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Methylrosanilinium Chloride 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).

(3) Zinc—To 0.10 g of Methylrosanilinium Chloride add 0.1 mL of sulfuric acid, and incinerate by ignition. After cooling, boil with 5 mL of dilute hydrochloric acid, 0.5 mL of dilute nitric acid and 4 mL of water, add 5 mL of ammonia TS, boil again, and filter. To the filtrate add 2 to 3 drops of sodium sulfide TS: no turbidity is produced.

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Methylrosanilinium Chloride, according to Method 3, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*>   Not more than 7.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*>   Not more than 1.5% (0.5 g).

Assay   Transfer about 0.4 g of Methylrosanilinium Chloride, accurately weighed, to a wide-mouthed, conical flask, add 25 mL of water and 10 mL of hydrochloric acid, dissolve, and add exactly 50 mL of 0.1 mol/L titanium (III) chloride VS while passing a stream of carbon dioxide through the flask. Heat to boil, and boil gently for 15 minutes, swirling the liquid frequently. Cool while passing a stream of carbon dioxide through the flask, titrate <*2.50*> the excess titanium (III) chloride with 0.05 mol/L ammonium iron (III) sulfate VS until a faint, red color is produced (indicator: 5 mL of ammonium thiocyanate TS). Perform a blank determination.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 20.40 mg of $C_{25}H_{30}ClN_3$

Containers and storage   Containers—Tight containers.

Methyl Salicylate

サリチル酸メチル
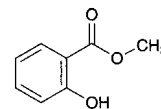
$C_8H_8O_3$: 152.15
Methyl 2-hydroxybenzoate
[*119-36-8*]

Methyl Salicylate contains not less than 98.0% of methyl salicylate ($C_8H_8O_3$).

Description   Methyl Salicylate is a colorless to pale yellow liquid. It has a strong, characteristic odor.
It is miscible with ethanol (95) and with diethyl ether.
It is very slightly soluble in water.
Specific gravity d_{20}^{20}: 1.182 – 1.192
Boiling point: 219 – 224°C

Identification   Shake 1 drop of Methyl Salicylate thoroughly with 5 mL of water for 1 minute, and add 1 drop of iron (III) chloride TS: a purple color develops.

Purity **(1)** Acidity—Shake 5.0 mL of Methyl Salicylate thoroughly with 25 mL of freshly boiled and cooled water and 1.0 mL of 0.1 mol/L sodium hydroxide VS for 1 minute, add 2 drops of phenol red TS, and titrate <*2.50*> with 0.1 mol/L hydrochloric acid VS until the red color disappears: not more than 0.45 mL of 0.1 mol/L sodium hydroxide VS is consumed.

(2) Heavy metals—Shake 10.0 mL of Methyl Salicylate thoroughly with 10 mL of water, add 1 drop of hydrochloric acid, and saturate with hydrogen sulfide by passing it through the mixture: neither the oily layer nor the aqueous layer shows a dark color.

Assay   Weigh accurately about 2 g of Methyl Salicylate, add an exactly measured 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and heat on a water bath for 2 hours under a reflux condenser. Cool, and titrate <*2.50*> the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS
= 76.08 mg of $C_8H_8O_3$

Containers and storage   Containers—Tight containers.

Compound Methyl Salicylate Spirit

複方サリチル酸メチル精

Method of preparation

Methyl Salicylate	40 mL
Capsicum Tincture	100 mL
d- or *dl*-Camphor	50 g
Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Spirits, with the above ingredients.

Description   Compound Methyl Salicylate Spirit is a red-

dish yellow liquid, having a characteristic odor and a burning taste.

Identification (1) Shake 1 mL of Compound Methyl Salicylate Spirit with 5 mL of dilute ethanol, and add 1 drop of iron (III) chloride TS: a purple color is produced (methyl salicylate).

(2) Shake thoroughly 1 mL of Compound Methyl Salicylate Spirit with 10 mL of chloroform, and use this solution as the sample solution. Dissolve 40 mg of methyl salicylate in 10 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 5 μL each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and chloroform (4:1) to a distance of about 10 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same Rf value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Containers and storage Containers—Tight containers.

Methyltestosterone

メチルテストステロン
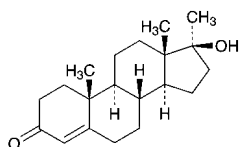
C$_{20}$H$_{30}$O$_2$: 302.45
17β-Hydroxy-17α-methylandrost-4-en-3-one
[*58-18-4*]

　Methyltestosterone, when dried, contains not less than 98.0% and not more than 102.0% of methyltestosterone (C$_{20}$H$_{30}$O$_2$).

Description Methyltestosterone occurs as white to pale yellow, crystals or 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 Methyltestosterone 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 Methyltestosterone 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 Methyltestosterone, 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 Methyltestosterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]$_D^{20}$: +79 – +85° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Melting point <2.60> 163 – 168°C

Purity Related substances—Dissolve 40 mg of Methyltestosterone in 2 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 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 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 10 hours).

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

Assay Weigh accurately about 20 mg each of Methyltestosterone and Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve each in methanol to make exactly 200 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 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 methyltestosterone to that of the internal standard.

$$\text{Amount (mg) of methyltestosterone (C}_{20}\text{H}_{30}\text{O}_2)$$
$$= M_S \times Q_T/Q_S$$

　M_S: Amount (mg) of Methyltestosterone RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).
Operating conditions—
　Detector: An ultraviolet absorption photometer (wavelength: 241 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 35°C.
　Mobile phase: A mixture of acetonitrile and water (11:9).
　Flow rate: Adjust so that the retention time of methyltestosterone 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 methyltestosterone 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 methyltestosterone to that of the internal standard is not more than 1.0%.

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

Methyltestosterone Tablets

メチルテストステロン錠

Methyltestosterone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$: 302.45).

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

Identification   To a portion of powdered Methyltestosterone Tablets, equivalent to 10 mg of Methyltestosterone, add 50 mL of acetone, shake for 30 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 10 mL of acetone, and use this solution as the sample solution. Separately, dissolve 10 mg of Methyltestosterone RS in 10 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 $10 \mu L$ 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) (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spot from the sample solution and the standard solution show the same Rf 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 Methyltestosterone Tablets add 5 mL of water to disintegrate, add 50 mL of methanol, and shake for 30 minutes. Add methanol to make exactly 100 mL, and centrifuge. Measure exactly V mL of the supernatant liquid, add methanol to make exactly V' mL of a solution containing about $10 \mu g$ of methyltestosterone ($C_{20}H_{30}O_2$) per ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, and dissolve in 5 mL of water and 50 mL of methanol, then 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. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at the wavelength of maximum absorption at about 241 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.25>.

$$\text{Amount (mg) of methyltestosterone } (C_{20}H_{30}O_2)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/10$$

M_S: Amount (mg) of Methyltestosterone RS taken

Dissolution <6.10>   When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of a solution prepared by dissolving 1 g of polysorbate 80 in water to make 5 L as the dissolution medium, the dissolution rate in 30 minutes of a 10-mg tablet is not less than 75% and that in 60 minutes of a 25-mg tablet is not less than 70%.

Start the test with 1 tablet of Methyltestosterone 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 $0.45 \mu m$. 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 $11 \mu g$ of methyltestosterone

($C_{20}H_{30}O_2$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Methyltestosterone RS, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 10 hours, and dissolve in ethanol (99.5) 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, at 249 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 methyltestosterone ($C_{20}H_{30}O_2$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of Methyltestosterone RS taken
C: Labeled amount (mg) of methyltestosterone ($C_{20}H_{30}O_2$) in 1 tablet

Assay   Weigh accurately the mass of not less than 20 Methyltestosterone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of methyltestosterone ($C_{20}H_{30}O_2$), add about 70 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, filter through a membrane filter (not exceeding $0.45 \mu m$ in pore size), and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Methyltestosterone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with $10 \mu L$ 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 methyltestosterone to that of the internal standard.

$$\text{Amount (mg) of methyltestosterone } (C_{20}H_{30}O_2)$$
$$= M_S \times Q_T/Q_S \times 5/4$$

M_S: Amount (mg) of Methyltestosterone RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 241 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5 \mu m$ in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: A mixture of acetonitrile and water (11:9).
Flow rate: Adjust so that the retention time of methyltestosterone 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 methyltestosterone 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 $10 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Meticrane

メチクラン
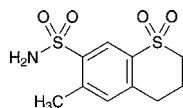
C$_{10}$H$_{13}$NO$_4$S$_2$: 275.34
6-Methylthiochromane-7-sulfonamide 1,1-dioxide
[*1084-65-7*]

Meticrane, when dried, contains not less than 98.0% of meticrane (C$_{10}$H$_{13}$NO$_4$S$_2$).

Description Meticrane occurs as white, crystals or crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, slightly soluble in acetonitrile and in methanol, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 234°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Meticrane in methanol (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 Meticrane, 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)** Ammonium ⟨*1.02*⟩—Perform the test with 0.10 g of Meticrane. Prepare the control solution with 3.0 mL of Standard Ammonium Solution (not more than 0.03%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Meticrane 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 Meticrane according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Meticrane in 50 mL of acetonitrile. To 5 mL of this solution add the mobile phase to make 25 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, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than meticrane from the sample solution is not larger than the peak area of meticrane from the standard solution.

Operating conditions 1—

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 water and acetonitrile (17:3).

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

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

System suitability 1—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane 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 10 mg each of Meticrane and caffeine in 100 mL of acetonitrile. To exactly 2 mL of this solution 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 1, caffeine and meticrane 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 1, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Operating conditions 2—

Detector, column, and column temperature: Proceed as directed in the operating conditions 1.

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

Flow rate: Adjust so that the retention time of meticrane is about 2 minutes.

Time span of measurement: About 10 times as long as the retention time of meticrane, beginning after the solvent peak.

System suitability 2—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane 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 20 mg each of Meticrane and methyl parahydroxybenzoate in 100 mL of acetonitrile. To exactly 2 mL of this solution 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 2, meticrane 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 10 μL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of meticrane 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 Meticrane, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, add 5 mL of water, and titrate ⟨2.50⟩ with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.
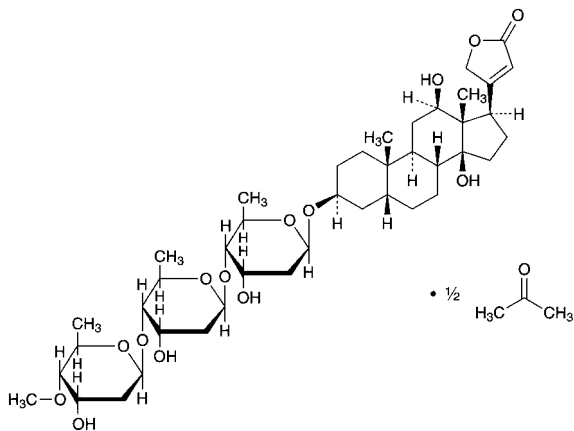
Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 27.54 mg of C$_{10}$H$_{13}$NO$_4$S$_2$

Containers and storage Containers—Well-closed contain-

ers.

Metildigoxin

メチルジゴキシン

$C_{42}H_{66}O_{14} \cdot \frac{1}{2}C_3H_6O$: 824.00
3β-[2,6-Dideoxy-4-*O*-methyl-β-D-*ribo*-hexopyranosyl-
$(1\rightarrow4)$-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-$(1\rightarrow4)$-
2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-12β,14-
dihydroxy-5β-card-20(22)-enolide—acetone (2/1)
[*30685-43-9*, acetone-free]

Metildigoxin contains not less than 96.0% and not more than 103.0% of metildigoxin ($C_{42}H_{66}O_{14}$. $\frac{1}{2}C_3H_6O$), calculated on the anhydrous basis.

Description   Metildigoxin occurs as a white to light yellowish white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, in pyridine and in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, and very slightly soluble in water.

It shows crystal polymorphism.

Identification **(1)**   Dissolve 2 mg of Metildigoxin in 2 mL of acetic acid (100), shake well with 1 drop of iron (III) chloride TS, and add gently 2 mL of sulfuric acid to divide into two layers: a brown color develops at the interface, and a deep blue color gradually develops in the acetic acid layer.

(2)   Dissolve 2 mg of Metildigoxin in 2 mL of 1,3-dinitrobenzene TS, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and shake: a purple color gradually develops, and changes to blue-purple.

(3)   Determine the absorption spectrum of a solution of Metildigoxin 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 Metildigoxin 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 Metildigoxin 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 Metildigoxin RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Metildigoxin and Metildigoxin RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_{546.1}^{20}$: $+22.0 - +25.5°$ (1 g calcu-

lated on the anhydrous basis, pyridine, 10 mL, 100 mm).

Purity **(1)**   Arsenic <1.11>—Prepare the test solution with 0.5 g of Metildigoxin according to Method 3, and perform the test (not more than 4 ppm).

(2)   Related substances—Dissolve 10 mg of Metildigoxin 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 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 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 2-butanone and chloroform (3:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly dilute sulfuric acid 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.

Acetone   Weigh accurately about 0.1 g of Metildigoxin, dissolve in exactly 2 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of acetone in a 50-mL volumetric flask containing about 10 mL of *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, then add *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 conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetone to that of the internal standard: the amount of acetone is between 2.0% and 5.0%.

$$\text{Amount (\%) of acetone} = M_S/M_T \times Q_T/Q_S$$

M_S: Amount (g) of acetone taken
M_T: amount (g) of Metildigoxin taken

Internal standard solution—A solution of *t*-butyl alcohol in *N*,*N*-dimethylformamide (1 in 2000).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column about 2 mm in inside diameter and 1 to 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).
Column temperature: A constant temperature between 170°C and 230°C.
Carrier gas: Nitrogen.
Flow rate: Adjust so that the retention time of acetone is about 2 minutes.
Selection of column: Proceed with 1 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of acetone and *t*-butyl alcohol in this order with the resolution between these peaks being not less than 2.0.

Water <2.48>   Not more than 3.0% (0.3 g, volumetric titration, direct titration).

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

Assay   Weigh accurately 0.1 g each of Metildigoxin and Metildigoxin RS (separately, determine the water <2.48> in the same manner as Metildigoxin), and dissolve each in methanol to make exactly 50 mL. Pipet 5 mL each of the solutions, add methanol to each to make exactly 100 mL, and use these solutions as the sample solution and the standard

solution, respectively. Pipet 5 mL each of the sample solution and standard solution, add 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS to each, shake well, add methanol to make exactly 25 mL, and allow to stand at $20 \pm 0.5°C$ for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution prepared by mixing 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS and adding methanol to make exactly 25 mL as the blank. Determine the maximum absorbances, A_T and A_S, of the subsequent solutions obtained from the sample solution and the standard solution, respectively, by measuring every 5 minutes, at 495 nm.

Amount (mg) of metildigoxin ($C_{42}H_{66}O_{14}.\frac{1}{2}C_3H_6O$)
$$= M_S \times A_T/A_S$$

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

Containers and storage   Containers—Tight containers.

Metoclopramide

メトクロプラミド
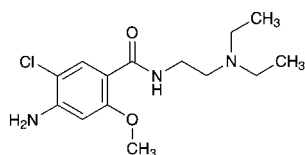
$C_{14}H_{22}ClN_3O_2$: 299.80
4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide
[*364-62-5*]

Metoclopramide, when dried, contains not less than 99.0% of metoclopramide ($C_{14}H_{22}ClN_3O_2$).

Description   Metoclopramide occurs as white, crystals or a crystalline powder, and is odorless.

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

It dissolves in dilute hydrochloric acid.

Identification   **(1)**   Dissolve 10 mg of Metoclopramide in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for Primary Aromatic Amines.

(2)   Dissolve 10 mg of Metoclopramide in 5 mL of dilute hydrochloric acid and 20 mL of water, and to 5 mL of this solution add 1 mL of Dragendorff's TS: a reddish orange precipitate is produced.

(3)   Dissolve 0.1 g of Metoclopramide in 1 mL of 1 mol/L hydrochloric acid TS, and dilute with water 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.

Melting point <2.60>   146 – 149°C

Purity   **(1)**   Clarity and color of solution—Dissolve 1.0 g of Metoclopramide in 10 mL of 1 mol/L hydrochloric acid

TS: the solution is clear and colorless.

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Metoclopramide as directed under 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>—Dissolve 1.0 g of Metoclopramide in 5 mL of 1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test (not more than 2 ppm).

(4)   Related substances—Dissolve 0.10 g of Metoclopramide in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of the sample solution, exactly measured, with 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 1-butanol and ammonia solution (28) (19:1) to a distance of about 10 cm. Dry the plate, first in air and then at 80°C for 30 minutes. 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, 3 hours).

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

Assay   Dissolve about 0.4 g of Metoclopramide, previously dried and accurately weighed, in 50 mL of acetic acid (100), add 5 mL of acetic anhydride, and warm for 5 minutes. Allow to cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform the blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
$$= 29.98 \text{ mg of } C_{14}H_{22}ClN_3O_2$$

Containers and storage   Containers—Well-closed containers.

Metoclopramide Tablets

メトクロプラミド錠

Metoclopramide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metoclopramide ($C_{14}H_{22}ClN_3O_2$: 299.80).

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

Identification   **(1)**   To a quantity of powdered Metoclopramide Tablets, equivalent to 50 mg of Metoclopramide, add 15 mL of 0.5 mol/L hydrochloric acid TS, and heat in a water bath at 70°C for 15 minutes while frequent shaking. After cooling, centrifuge for 10 minutes, and to 5 mL of the supernatant liquid add 1 mL of 4-dimethylaminobenzaldehyde-hydrochloric acid TS: a yellow color develops.

(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 270 nm and 274 nm, and between 306 nm and 310 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 Metoclopramide Tablets add 10 mL of 0.1 mol/L hydrochloric acid TS, disperse the particles with the aid of ultrasonic waves, then add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 12 μg of metoclopramide ($C_{14}H_{22}ClN_3O_2$), and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 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. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of metoclopramide } (C_{14}H_{22}ClN_3O_2)$$
$$= M_S \times A_T/A_S \times V/1000$$

M_S: Amount (mg) of metoclopramide for assay taken

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

Assay Weigh accurately not less than 20 Metoclopramide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of metoclopramide ($C_{14}H_{22}ClN_3O_2$), add 300 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Centrifuge for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 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. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.
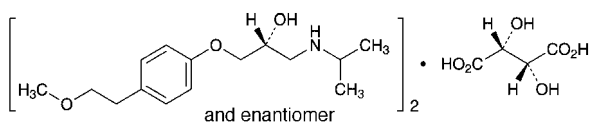
$$\text{Amount (mg) of metoclopramide } (C_{14}H_{22}ClN_3O_2)$$
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of metoclopramide for assay taken

Containers and storage Containers—Tight containers.

Metoprolol Tartrate

メトプロロール酒石酸塩

and enantiomer

$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$: 684.81
(2*RS*)-1-[4-(2-Methoxyethyl)phenoxy]-3-
[(1-methylethyl)amino]propan-2-ol hemi-(2*R*,3*R*)-tartrate
[*56392-17-7*]

Metoprolol Tartrate, when dried, contains not less than 99.0% and not more than 101.0% of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$].

Description Metoprolol Tartrate occurs as a white crystal-line powder.

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

Optical rotation $[\alpha]_D^{20}$: +7.0 – +10.0° (after drying, 1 g, water, 50 mL, 100 mm).

It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Metoprolol Tartrate in ethanol (95) (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 Metoprolol Tartrate, previously dried, 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. If any difference appears between the spectra, recrystallize Metoprolol Tartrate from a solution in acetone (23 in 1000), filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Metoprolol Tartrate (1 in 5) responds to the Qualitative Tests <1.09> (1) for tartrate.

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Metoprolol Tartrate in 10 mL of water is between 6.0 and 7.0.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Metoprolol Tartrate 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) Related substances—Dissolve 0.10 g of Metoprolol Tartrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add 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 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After saturating the plate with the atmosphere by allowing to stand in a developing vessel, which contains the developing solvent and a glass vessel containing ammonia water (28), develop with the developing solvent, a mixture of ethyl acetate and methanol (4:1), to a distance of about 12 cm, and air-dry the plate. Allow to stand the plate in an iodine vapors until the spot with the standard solution appears obviously: the spot other than the principal spot and other than the spot on the original point with the sample solution is not more than three spots, 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, in vacuum, 60°C, 4 hours).

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

Assay Weigh accurately about 0.5 g of Metoprolol Tartrate, 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.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 34.24 \text{ mg of } (C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$$

Containers and storage Containers—Well-closed containers.

Metoprolol Tartrate Tablets

メトプロロール酒石酸塩錠

Metoprolol Tartrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$: 684.81].

Method of preparation   Prepare as directed under Tablets, with Metoprolol Tartrate.

Identification   To an amount of powdered Metoprolol Tartrate Tablets, equivalent to 10 mg of Metoprolol Tartrate, add 100 mL of ethanol (95), shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 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 Metoprolol Tartrate Tablets add 1 mL of water for every 10 mg of Metoprolol Tartrate, shake for 20 minutes, then add 75 mL of ethanol (95), shake for 15 minutes, add ethanol (95) to make exactly 100 mL, and centrifuge. Pipet V mL of the supernatant liquid, add ethanol (95) to make exactly V' so that each mL contains about 0.1 mg of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$], and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 5 mL of water, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add ethanol (95) 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 276 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using ethanol (95) as the blank.

Amount (mg) of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$]
   $= M_S \times A_T/A_S \times V'/V \times 1/5$

M_S: Amount (mg) of metoprolol tartrate 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 Metoprolol Tartrate Tablets is not less than 80%.

Start the test with 1 tablet of Metoprolol Tartrate 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 22 μg of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$], and use this solution as the sample solution. Separately, weigh accurately about 56 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in water to make exactly 200 mL. Pipet 8 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 metoprolol in each solution.

Dissolution rate (%) with respect to the labeled amount of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$]
   $= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of metoprolol tartrate for assay taken
C: Labeled amount (mg) of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$] 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 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metoprolol are not less than 2000 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 metoprolol is not more than 2.0%.

Assay   Weigh accurately the mass of not less than 20 Metoprolol Tartrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.12 g of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$], add 60 mL of a mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) and exactly 10 mL of the internal standard solution, shake for 15 minutes, and add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.12 g of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 60 mL of the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1), add exactly 10 mL of the internal standard solution, then add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) 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 metoprolol to that of the internal standard.

Amount (mg) of metoprolol tartrate [$(C_{15}H_{25}NO_3)_2.C_4H_6O_6$]
   $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of metoprolol tartrate for assay taken

*Internal standard solution—*A solution of ethyl parahydroxybenzoate in the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) (1 in 500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 274 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 14.0 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust to pH 3.2 with diluted perchloric acid (17 in 2000). To 750 mL of this solution add 250 mL of acetonitrile.
Flow rate: Adjust so that the retention time of metoprolol 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, metoprolol 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 metoprolol to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Metronidazole

メトロニダゾール
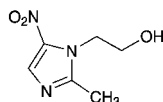
C$_6$H$_9$N$_3$O$_3$: 171.15
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethanol
[*443-48-1*]

Metronidazole, when dried, contains not less than 99.0% and not more than 101.0% of metronidazole (C$_6$H$_9$N$_3$O$_3$).

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

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and slightly soluble in water.

It dissolves in dilute hydrochloric acid.

It is colored to yellow-brown by light.

Identification   (1)   Determine the absorption spectrum of a solution of Metronidazole 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 Metronidazole 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>   159 – 163°C

Purity   (1)   Heavy metals <1.07>—Proceed with 1.0 g of Metronidazole 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)   2-Methyl-5-nitroimidazol—Dissolve 0.10 g of Metronidazole in acetone to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in acetone to make exactly 20 mL, then pipet 5 mL of this 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 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the

standard 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, silica gel, 24 hours).

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

Assay   Weigh accurately about 0.2 g of Metronidazole, previously dried, and dissolve in 30 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 0.5 mL of *p*-naphtholbenzein TS) until the color of the solution changes from orange-yellow to green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.12 mg of C$_6$H$_9$N$_3$O$_3$

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

Metronidazole Tablets

メトロニダゾール錠

Metronidazole Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metronidazole (C$_6$H$_9$N$_3$O$_3$: 171.15).

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

Identification   (1)   To an amount of powdered Metronidazole Tablets, equivalent to 0.1 g of Metronidazole, add 100 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand for 30 minutes with occasional stirring. Then, shake vigorously, and centrifuge a part of this solution. To 1 mL of the supernatant liquid 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 275 nm and 279 nm.

(2)   Shake vigorously a quantity of powdered Metronidazole Tablets, equivalent to 0.20 g of Metronidazole, with 20 mL of acetone for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.10 g of metronidazole in 10 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 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 immediately with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the principal spots obtained from the sample solution and the standard solution is 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 Metronidazole Tablets add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 25 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter the solution through a membrane filter with pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter,

proceed as directed in the Assay.

$$\text{Amount (mg) of metronidazole } (C_6H_9N_3O_3)$$
$$= M_S \times A_T/A_S \times 10$$

M_S: Amount (mg) of metronidazole 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 Metronidazole Tablets is not less than 70%.

Start the test with 1 tablet of Metronidazole 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 metronidazole ($C_6H_9N_3O_3$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of metronidazole for assay, previously dried in vacuum with silica gel for 24 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, at 320 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Dissolution rate (\%) with respect to the labeled amount}$$
$$\text{of metronidazole } (C_6H_9N_3O_3)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of metronidazole for assay taken
C: Labeled amount (mg) of metronidazole ($C_6H_9N_3O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Metronidazole Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of metronidazole ($C_6H_9N_3O_3$), add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 10 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter this solution through a membrane filter with pore size of 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of metronidazole for assay, previously dried in vacuum on silica gel for 24 hours, dissolve in the mixture of water and methanol (4:1) 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 metronidazole in each solution.

$$\text{Amount (mg) of metronidazole } (C_6H_9N_3O_3)$$
$$= M_S \times A_T/A_S \times 10$$

M_S: Amount (mg) of metronidazole for assay taken

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 320 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 (4:1).
Flow rate: Adjust so that the retention time of metronidazole 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 metronidazole 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 metronidazole is not more than 1.0%.

Containers and storage Containers—Tight containers.

Metyrapone

メチラポン
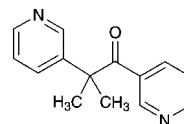
$C_{14}H_{14}N_2O$: 226.27
2-Methyl-1,2-di(pyridin-3-yl)propan-1-one
[*54-36-4*]

Metyrapone, when dried, contains not less than 98.0% of metyrapone ($C_{14}H_{14}N_2O$).

Description Metyrapone occurs as a white to pale yellow crystalline powder. It has a characteristic odor and a bitter taste.

It is very soluble in methanol, in ethanol (95), in acetic anhydride, in chloroform, in diethyl ether and in nitrobenzene, and sparingly soluble in water.

It dissolves in 0.5 mol/L sulfuric acid TS.

Identification (1) Mix 5 mg of Metyrapone with 10 mg of 1-chloro-2,4-dinitrobenzene, melt by gently heating for 5 to 6 seconds, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Determine the absorption spectrum of a solution of Metyrapone in 0.5 mol/L sulfuric 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.

Melting point ⟨*2.60*⟩ 50 – 54°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Metyrapone in 5 mL of methanol: the solution is clear and colorless to pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Metyrapone 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 Metyrapone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Metyrapone in 5 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 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 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 chloroform and methanol (15:1) to a distance of about 10 cm, and air-dry the plate for about 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm): 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 than 0.5% (1 g, in vacuum, silica gel, 24 hours).

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

Assay   Weigh accurately about 0.2 g of Metyrapone, previously dried, dissolve in 10 mL of nitrobenzene and 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.
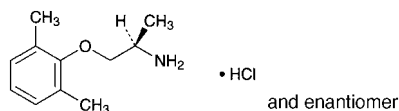
Each mL of 0.1 mol/L perchloric acid VS
= 11.31 mg of $C_{14}H_{14}N_2O$

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

Mexiletine Hydrochloride

メキシレチン塩酸塩

and enantiomer

$C_{11}H_{17}NO.HCl$: 215.72
(1*RS*)-2-(2,6-Dimethylphenoxy)-1-methylethylamine monohydrochloride
[*5370-01-4*]

Mexiletine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of mexiletine hydrochloride ($C_{11}H_{17}NO.HCl$).

Description   Mexiletine Hydrochloride occurs as a white powder.

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

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Mexiletine Hydrochloride (1 in 20) shows no optical rotation.

Mexiletine Hydrochloride shows crystal polymorphism.

Identification   **(1)**   Determine the absorption spectrum of a solution of Mexiletine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mexiletine 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 Mexiletine Hydrochloride, previously dried, 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 dried Mexiletine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Mexiletine Hydrochloride from methanol (95), filter, dry the crystals, and perform the test with the crystals.

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

pH <2.54>   Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the pH of this solution is between 3.8 and 5.8.

Melting point <2.60>   200 – 204°C

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

(2)   Heavy Metals <1.07>—Proceed with 2.0 g of Mexiletine 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)   Related substances—Dissolve 20 mg of Mexiletine 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 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. Determine each peak area of both solutions by the automatic integration method: each peak area other than mexiletine from the sample solution is not larger than the peak area of mexiletine 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 so that the peak height of mexiletine obtained from 20 μL of the standard solution is between 5 mm and 10 mm.

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

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 20 mg each of Mexiletine Hydrochloride and Mexiletine Hydrochloride RS, each previously dried, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 5 mL each of these solutions, 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 mexiletine to that of the internal standard, respectively.

Amount (mg) of mexiletine hydrochloride ($C_{11}H_{17}NO.HCl$)
= $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Mexiletine Hydrochloride RS taken

Internal standard solution—A solution of phenetylamine hydrochloride in the mobile phase (3 in 5000).
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 octylsilanized silica gel for liquid chromatography (about 7 μm in particle diameter).

Column temperature: A constant temperature of about

30°C.

Mobile phase: Dissolve 2.5 g of sodium lauryl sulfate and 3 g of sodium dihydrogen phosphate dihydrate in 600 mL of water, and add 420 mL of acetonitrile.

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

Selection of column: Proceed with 20 μL of the standard solution under the above conditions, and calculate the resolution. Use a column giving elution of the internal standard and mexiletine in this order with the resolution between these peaks being not less than 9.

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

Miconazole

ミコナゾール

and enantiomer
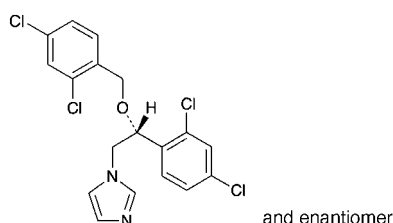
$C_{18}H_{14}Cl_4N_2O$: 416.13
1-[(2*RS*)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole
[*22916-47-8*]

Miconazole, when dried, contains not less than 98.5% of miconazole ($C_{18}H_{14}Cl_4N_2O$).

Description　Miconazole occurs as a white to pale yellowish white crystalline powder.

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

A solution of Miconazole in methanol (1 in 20) shows no optical rotation.

Identification　**(1)**　Determine the absorption spectrum of a solution of Miconazole in methanol (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 Miconazole, 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>　84 – 87°C

Purity　**(1)**　Heavy metals <1.07>—Proceed with 1.0 g of Miconazole 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 Miconazole according to Method 3, and perform the test (not more than 2 ppm).

(3)　Related substances—Dissolve 0.10 g of Miconazole 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 20 mL. 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 50 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, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 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, silica gel, 60%, 3 hours).

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

Assay　Weigh accurately about 0.3 g of Miconazole, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of *p*-naphtholbenzein TS) until the color of the solution changes from light yellow-brown to light yellow-green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.61 mg of $C_{18}H_{14}Cl_4N_2O$

Containers and storage　Containers—Tight containers.

Miconazole Nitrate

ミコナゾール硝酸塩

• HNO₃

and enantiomer
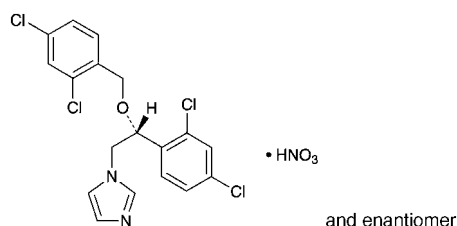
$C_{18}H_{14}Cl_4N_2O.HNO_3$: 479.14
1-[(2*RS*)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole mononitrate
[*22832-87-7*]

Miconazole Nitrate, when dried, contains not less than 98.5% of miconazole nitrate ($C_{18}H_{14}Cl_4N_2O$. HNO₃).

Description　Miconazole Nitrate occurs as a white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (95), in acetone and in acetic acid (100), and very slightly soluble in water and in diethyl ether.

Melting point: about 180°C (with decomposition).

Identification　**(1)**　To 2 mL of a solution of Miconazole Nitrate in methanol (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

(2)　Determine the absorption spectrum of a solution of Miconazole Nitrate in methanol (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.

(3) Perform the test with a solution of Miconazole Nitrate in methanol (1 in 100) as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(4) A solution of Miconazole Nitrate in methanol (1 in 100) responds to the Qualitative Tests <*1.09*> for nitrate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Miconazole Nitrate in 100 mL of methanol: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.10 g of Miconazole Nitrate in 6 mL of dilute nitric acid and *N,N*-dimethylformamide 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 *N,N*-dimethylformamide to make 50 mL (not more than 0.09%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Miconazole Nitrate 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) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Miconazole Nitrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Miconazole Nitrate 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 20 mL, 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 50 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, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate in iodine vapor for 20 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, silica gel, 60°C, 3 hours).

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

Assay   Weigh accurately about 0.35 g of Miconazole Nitrate, 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.
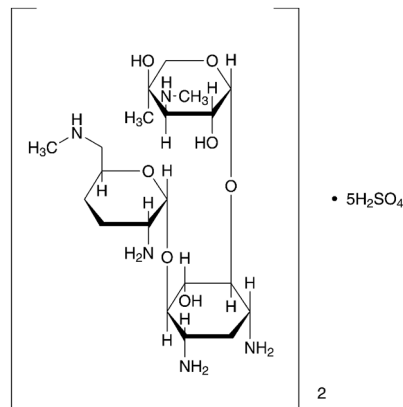
Each mL of 0.1 mol/L perchloric acid VS
= 47.91 mg of $C_{18}H_{14}Cl_4N_2O.HNO_3$

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

Micronomicin Sulfate

ミクロノマイシン硫酸塩

$(C_{20}H_{41}N_5O_7)_2.5H_2SO_4$: 1417.53
2-Amino-2,3,4,6-tetradeoxy-6-methylamino-α-D-*erythro*-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine hemipentasulfate
[*52093-21-7*, Micronomicin]

Micronomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora sagamiensis*.

It contains not less than 590 μg (potency) and not more than 660 μg (potency) per mg, calculated on the anhydrous basis. The potency of Micronomicin Sulfate is expressed as mass (potency) of micronomicin ($C_{20}H_{41}N_5O_7$: 463.57).

Description   Micronomicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification (1) Dissolve 50 mg each of Micronomicin Sulfate and Micronomicin Sulfate RS in 10 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 5 μL 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), 1-butanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spots obtained from the sample solution and the standard solution are red-purple to red-brown and their *R*f values are the same.

(2) To 5 mL of a solution of Micronomicin Sulfate (1 in 100) add 1 mL of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: + 110 – + 130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <*2.54*>   The pH of a solution obtained by dissolving 1.0 g of Micronomicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.5 g of Micronomicin Sulfate 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 Micronomicin 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).

(3) Related substances—Dissolve 0.40 g of Micronomicin 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 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 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), 1-butanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°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*> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) 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—*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 Micronomicin Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 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 for antibiotics (pH 8.0) to make solutions so that each mL contains 2 μg (potency) and 0.5 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 Micronomicin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 20 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 2 μg (potency) and 0.5 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.

Midecamycin

ミデカマイシン
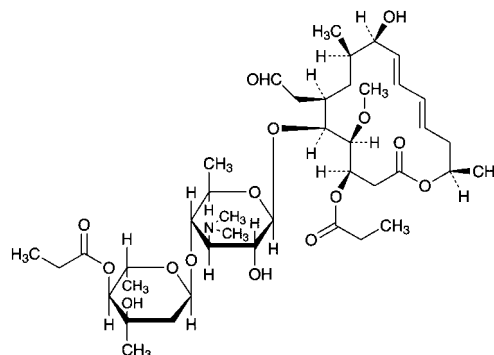
C$_{41}$H$_{67}$NO$_{15}$: 813.97
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-
5-[2,6-Dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-
hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-
glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-
8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide
[*35457-80-8*]

Midecamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces mycarofaciens*.

It contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin is expressed as mass (potency) of midecamycin (C$_{41}$H$_{67}$NO$_{15}$).

Description Midecamycin occurs as a white crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Midecamycin 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 Midecamycin 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 Midecamycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Midecamycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 153 – 158°C

Purity Heavy metals <*1.07*>—Proceed with 1.0 g of Midecamycin 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).

Loss on drying <*2.41*> Not more than 2.0% (1.0 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

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—*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 Midecamycin RS, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add water 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 0.1 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 Midecamycin, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, and add water to make exactly 50 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.

Midecamycin Acetate

ミデカマイシン酢酸エステル
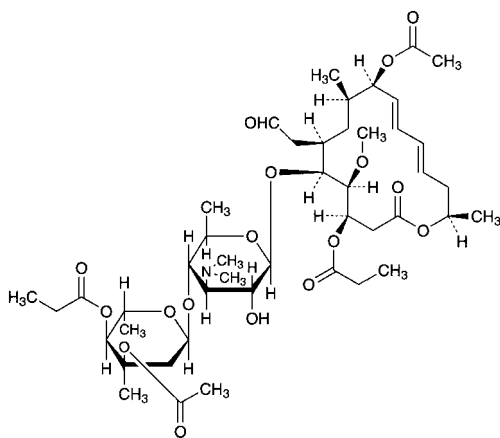
C$_{45}$H$_{71}$NO$_{17}$: 898.04
(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-9-Acetoxy-5-[3-*O*-acetyl-2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-3-propioyloxyhexadeca-10,12-dien-15-olide
[*55881-07-7*]

Midecamycin Acetate is a derivative of midecamycin.

It contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin Acetate is expressed as mass (potency) of midecamycin acetate (C$_{45}$H$_{71}$NO$_{17}$).

Description Midecamycin Acetate occurs as white, crystals or crystalline powder.

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

Identification (1) Determine the absorption spectrum of a solution of Midecamycin Acetate 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 Midecamycin Acetate 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 Midecamycin 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 spectrum of dried Midecamycin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin Acetate 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 2.0% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

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—*Kocuria rhizophila* ATCC 9341

(ii) Culture medium—Use the medium i in 3) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Midecamycin Acetate RS, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°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 4.5) 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 Midecamycin Acetate, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) 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.

Miglitol

ミグリトール
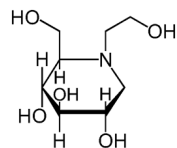
C$_8$H$_{17}$NO$_5$: 207.22
(2R,3R,4R,5S)-1-(2-Hydroxyethyl)-2-
(hydroxymethyl)piperidine-3,4,5-triol
[*72432-03-2*]

Miglitol contains not less than 98.0% and not more than 102.0% of miglitol (C$_8$H$_{17}$NO$_5$), calculated on the dried basis.

Description Miglitol is a white to pale yellowish white powder.

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

Identification **(1)** Determine the infrared absorption spectrum of Miglitol, 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 Miglitol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg each of Miglitol and Miglitol 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 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, ethyl acetate and diluted ammonia solution (28) (9 in 10) (2:2:1) to a distance of about 17 cm, and dry the plate at 105°C. Allow the plate to stand in iodine vapor: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a brown color and the same Rf value.

Optical rotation <2.49> $[\alpha]_D^{20}$: − 7.3 – − 8.3°(1.2 g calculated on the dried basis, water, 50 mL, 100 mm).

Melting point <2.60> 144 – 147°C

Purity **(1)** Clarity and color of solution—Dissolve 2.5 g of Miglitol in 50 mL of water, and use this solution as the test solution. Determine the turbidity of the test solution as directed under Turbidity Measurement <2.61>: it exhibits no more turbidity than Reference suspension II, and has no more color than the following control solution.

Control solution: To a mix of 0.3 mL of Cobalt (II) Chloride CS and 1.2 mL of Iron (III) Chloride CS add 38.5 mL of diluted hydrochloric acid (1 in 100).

(2) Heavy metals—Dissolve 2.5 g of Miglitol in 25 mL of water, and use this solution as the sample solution. Separately, to 10 mL of a solution obtained by diluting Standard Lead Stock Solution to 50-fold with water before use, add 2 mL of the sample solution, and use this solution as the control solution. To 12 mL of the sample solution and the control solution add 2 mL of hydrochloric acid-ammonium acetate buffer solution (pH 3.5) and 1.2 mL of thioacetamide TS, mix, allow to stand for 2 minutes, and observe vertically

or horizontally both Nessler tubes against a white background: the color obtained with the sample solution is not more intense than that obtained with the control solution (not more than 20 ppm).

(3) Related substances—Dissoble 0.19 g of Miglitol in 50 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 each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peaks having the relative retention time of about 0.9 and about 1.5 to miglitol is not more than 0.2%, and the amount of the peaks other than miglitol and the peaks mentioned above is not more than 0.1%. The total amount of the peaks other than miglitol is not more than 0.5%. For the area of the peak, having the relative retention time of about 1.5 to miglitol, multiply the relative response factor 4.1.
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 3 times as long as the retention time of miglitol, beginning after the solvent peak.
System suitability—

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 miglitol 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 miglitol 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of miglitol is not more than 5.0%.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, 60°C, 6 hours).

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

Assay Weigh accurately about 50 mg each of Miglitol and Miglitol RS (separately determine the loss on drying <2.41> under the same conditions as Miglitol), dissolve each 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 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 miglitol in each solution.

Amount (mg) of miglitol (C$_8$H$_{17}$NO$_5$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Miglitol RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wave length: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with pentaethylenehexaaminated polyvinyl alcohol polymer beads for liquid chromatog-

raphy (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 0.6 g of potassium dihydrogen phosphate and 0.28 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL. To 300 mL of this solution add 900 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of miglitol 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 miglitol 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 conditions, the relative standard deviation of the peak area of miglitol is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Migrenin

ミグレニン

Migrenin is composed of 90 parts of antipyrine, 9 parts of caffeine, and 1 part of citric acid in mass.

Migrenin, when dried, contains not less than 87.0% and not more than 93.0% of antipyrine ($C_{11}H_{12}N_2O$: 188.23) and not less than 8.6% and not more than 9.5% of caffeine ($C_8H_{10}N_4O_2$: 194.19).

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

It is very soluble in water, freely soluble in ethanol (95) and in chloroform, and slightly soluble in diethyl ether.

The pH of a solution of 1.0 g of Migrenin in 10 mL of water is between 3.0 and 4.0.

It is affected by moisture and light.

Identification   (1)   To 5 mL of a solution of Migrenin (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

(2)   To 5 mL of a solution of Migrenin (1 in 50) add 1 drop of hydrochloric acid and 0.2 mL of formaldehyde solution, heat in a water bath for 30 minutes, add an excess of ammonia TS, and filter. Acidify the filtrate with hydrochloric acid, shake with 3 mL of chloroform, and separate the chloroform layer. Evaporate the chloroform solution on a water bath, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid to the residue, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: a red-purple color develops, disappearing on the addition of 2 to 3 drops of sodium hydroxide TS.

(3)   A solution of Migrenin (1 in 10) responds to the Qualitative Tests <1.09> for citrate.

Melting point <2.60>   104 – 110°C

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Migrenin in 40 mL of water: the solution is clear and colorless to pale yellow.

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Migrenin 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 0.5% (1 g, in vacuum, silica gel, 4 hours).

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

Assay   (1)   Antipyrine—Weigh accurately about 0.25 g of Migrenin, previously dried in an iodine flask, dissolve in 25 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Add 15 mL of chloroform to dissolve the precipitate so obtained, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

$$\text{Each mL of 0.05 mol/L iodine VS} = 9.411 \text{ mg of } C_{11}H_{12}N_2O$$

(2)   Caffeine—To about 1 g of Migrenin, previously dried and accurately weighed, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of Caffeine RS, previously dried at 80°C for 4 hours, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 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 caffeine to that of the internal standard.

$$\text{Amount (mg) of caffeine } (C_8H_{10}N_4O_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Caffeine RS taken

*Internal standard solution—*A solution of ethenzamide in chloroform (1 in 50).
Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 210 cm in length, packed with siliceous earth for gas chromatography (180 to 250 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 15%.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethenzamide is about 4 minutes.

System suitability—

System performance: Dissolve 0.9 g of antipyrine and 0.09 g of caffeine in 10 mL of chloroform. When the procedure is run with 1 μL of this solution under the above operating conditions, caffeine and antipyrine 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 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of caffeine to that of the internal standard is not more than 1.0%.

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

Minocycline Hydrochloride

ミノサイクリン塩酸塩
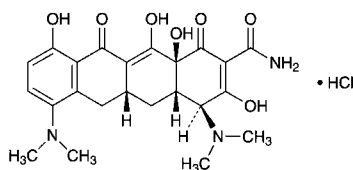
C$_{23}$H$_{27}$N$_3$O$_7$.HCl: 493.94
(4S,4aS,5aR,12aS)-4,7-Bis(dimethylamino)-
3,10,12,12a-tetrahydroxy-1,11-dioxo-
1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide
monohydrochloride
[*13614-98-7*]

Minocycline Hydrochloride is the hydrochloride of a derivative of tetracycline.

It contains not less than 890 μg (potency) and not more than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Minocycline Hydrochloride is expressed as mass (potency) of minocycline (C$_{23}$H$_{27}$N$_3$O$_7$: 457.48).

Description Minocycline Hydrochloride occurs as a yellow crystalline powder.

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

Identification (1) Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Minocycline 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 Minocycline 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 Minocycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

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

pH <2.54> Dissolve 1.0 g of Minocycline Hydrochloride in 100 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 Minocycline Hydrochloride in 100 mL of water: the solution is clear, and when the test is performed within 1 hour after preparation of this solution, the absorbance of the solution at 560 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.06.

(2) Heavy metals <1.07>—Proceed with 0.5 g of Minocycline Hydrochloride 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).

(3) Related substances—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test, immediately after the preparation of the sample solution, with 20 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. Calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2%, the amount of each peak other than minocycline and epiminocycline is not more than 1.0%, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0%.

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 minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition.

Time span of measurement: About 2.5 times as long as the retention time of minocycline, 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 sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48> Not less than 4.3% and not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Minocycline Hydrochloride and Minocycline Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and 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 minocycline in each solution.

Amount [μg (potency)] of minocycline (C$_{23}$H$_{27}$N$_3$O$_7$)
   $= M_S \times A_T/A_S \times 1000$

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

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 octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Adjust to pH 6.5 of a mixture of a solution of ammonium oxalate monohydrate (7 in 250), *N,N*-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) with tetrabutylammonium hydroxide TS.

Flow rate: Adjust so that the retention time of minocy-

cline is about 12 minutes.

System suitability—

System performance: Dissolve 50 mg of Minocycline Hydrochloride in 25 mL of water. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, epiminocycline and minocycline 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 peak areas of minocycline is not more than 1.0%.

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

Minocycline Hydrochloride for Injection

注射用ミノサイクリン塩酸塩

Minocycline 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 minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Method of preparation   Prepare as directed under Injections, with Minocycline Hydrochloride.

Description   Minocycline Hydrochloride for Injection occurs as a yellow to yellow-brown, powder or flakes.

Identification   Dissolve 4 mg of Minocycline Hydrochloride for Injection in 250 mL of a solution of hydrochloric acid in methanol (19 in 20,000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

pH <2.54>   The pH of a solution, prepared by dissolving an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride, in 10 mL of water is 2.0 to 3.5.

Purity   Related substances—Conduct this procedure rapidly after the preparation of the sample solution. Take an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride, dissolve in the mobile phase to make 100 mL. To 25 mL of this solution, add the mobile phase to make 50 mL, 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 each peak area by the automatic integration method. Calculate the amounts of each peak by the area percentage method: the amount of epiminocycline, having the relative retention time of about 0.83 to minocycline, is not more than 6.0%.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

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

Test for required detectability: Pipet 2 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48>   Weigh accurately the mass of the content of one container of Minocycline Hydrochloride for Injection, dissolve in exactly 2 mL of methanol for water determination, and perform the test with exactly 1 mL of this solution as directed in the Volumetric titration (back titration): not more than 3.0%.

Bacterial endotoxins <4.01>   Less than 1.25 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 Minocycline Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of Minocycline Hydrochloride, dissolve in the mobile phase to make exactly 100 mL. Pipet 25 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Minocycline Hydrochloride.

Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$)
$= M_S \times A_T/A_S \times 4$

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

Containers and storage   Containers—Hermetic containers.

Minocycline Hydrochloride Tablets

ミノサイクリン塩酸塩錠

Minocycline Hydrochloride Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of Minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Method of preparation   Prepare as directed under Tablets, with Minocycline Hydrochloride.

Identification To a quantity of powdered Minocycline Hydrochloride Tablets, equivalent to 10 mg (potency) of Minocycline Hydrochloride, add 625 mL of a solution of hydrochloric acid in methanol (19 in 20,000), shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

Purity Related substances—Conduct this procedure rapidly after preparation of the sample solution. Powder not less than 5 Minocycline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to 50 mg (potency) of Minocycline Hydrochloride, add 60 mL of the mobile phase, shake vigorously, and add the mobile phase to make 100 mL. Centrifuge this solution, and use the supernatant liquid 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 each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the amount of the peak of epiminocycline, having the relative retention time of about 0.83 to minocycline, 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 under Minocycline Hydrochloride.

Time span of measurement: About 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

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

Test for required detectability: To 2 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 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of minocycline 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48> Not more than 12.0% (0.5 g of powdered Minocycline Hydrochloride Tablets, volumetric titration, back titration).

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 Minocycline Hydrochloride Tablets add 60 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly V mL so that each mL contains about 0.5 mg (potency) of Minocycline Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$)
= $M_S \times A_T/A_S \times V/50$

M_S: Amount [mg (potency)] of Minocycline 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 Minocycline Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Minocycline 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 9 μg (potency) of Minocycline Hydrochloride, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 30 mg (potency), 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 the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 348 nm.

Dissolution rate (%) with respect to the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount [mg (potency)] of Minocycline Hydrochloride RS taken
C: Labeled amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$) in 1 tablet

Assay To a number of Minocycline Hydrochloride Tablets, equivalent to about 1 g (potency) of Minocycline Hydrochloride, add 120 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly 200 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride RS, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Minocycline Hydrochloride.

Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$)
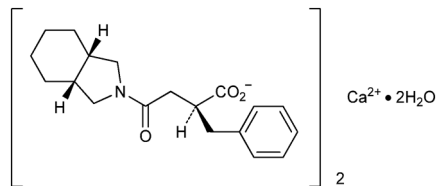= $M_S \times A_T/A_S \times 40$

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

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

Mitiglinide Calcium Hydrate

ミチグリニドカルシウム水和物

C$_{38}$H$_{48}$CaN$_2$O$_6$.2H$_2$O: 704.91
Monocalcium bis{(2S)-2-benzyl-4-[(3aR,7aS)-
octahydroisoindol-2-yl]-4-oxobutanoate} dihydrate
[207844-01-7]

Mitiglinide Calcium Hydrate contains not less than
98.0% and not more than 102.0% of mitiglinide cal-
cium hydrate (C$_{38}$H$_{48}$CaN$_2$O$_6$.2H$_2$O).

Description   Mitiglinide Calcium Hydrate occurs as a white
powder.

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

It shows crystal polymorphism.

Identification   (1)   Determine the absorption spectrum of a
solution of Mitiglinide Calcium Hydrate in methanol (1 in
1000) as directed under Ultraviolet-visible Spectrophotome-
try <2.24>, and compare the spectrum with the Reference
Spectrum or the spectrum of a solution of Mitiglinide Cal-
cium RS prepared in the same manner as the sample solu-
tion: both spectra exhibit similar intensities of absorption at
the same wavelengths.

(2)   Determine the infrared absorption spectrum of
Mitiglinide Calcium Hydrate as directed in the paste method
under Infrared Spectrophotometry <2.25>, and compare the
spectrum with the Reference Spectrum or the spectrum of
Mitiglinide Calcium RS: both spectra exhibit similar intensi-
ties of absorption at the same wave numbers.

(3)   To 0.5 g of Mitiglinide Calcium Hydrate add 3 mL of
1 mol/L hydrochloric acid TS and 5 mL of diethyl ether,
shake, then separate the aqueous layer, and neutralize with
ammonia TS: the solution responds to the Qualitative Tests
<1.09> (2) for calcium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: + 8.4 – + 9.0°(0.38 g calcu-
lated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity   (1)   Heavy metals   <1.07>—Place   1.0 g   of
Mitiglinide Calcium Hydrate in a crucible, cover the crucible
loosely, and ignite at a low temperature until charred. After
cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid
to the content of the crucible, heat carefully until white
fumes are no longer evolved, and ignite between 500 and
600°C. After cooling, moisten the residue with a little
amount of sulfuric acid, and incinerate again by ignition.
After cooling, add 2 mL of hydrochloric acid, evaporate to
dryness on a water bath, moisten the residue with 3 drops of
hydrochloric acid, add 10 mL of boiling water, and heat for
2 minutes. Treat this solution with ultrasonic waves, add 1
drop of phenolphthalein TS, drop ammonia TS until a slight
red color develops, add 2 mL of dilute acetic acid, transfer
to a centrifuge tube, centrifuge, and take the supernatant
liquid. Wash the residue in the crucible with 15 mL of water,
transfer to the former centrifuge tube, treat with ultrasonic
waves, centrifuge, and take the supernatant liquid. Repeat
this operation with 15 mL of water in addition. Combine all

the supernatant liquid obtained, put in a Nessler tube, and
add water to make 50 mL. Perform the test using this solu-
tion as the test solution. Prepare the control solution with
2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2)   Related substances—To 0.10 g of Mitiglinide Cal-
cium Hydrate add a mixture of water and acetonitrile (2:1),
dissolve by treating with ultrasonic waves while occasional
shaking, add the mixture of water and acetonitrile (2:1) to
make 100 mL, and use this solution as the sample solution.
Pipet 2 mL of the sample solution, add the mixture of water
and acetonitrile (2:1) to make exactly 50 mL. Pipet 2.5 mL
of this solution, add the mixture of water and acetonitrile
(2:1) to make exactly 20 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 fol-
lowing conditions, and determine each peak area by the au-
tomatic integration method: the area of the peak other than
mitiglinide obtained from the sample solution is not larger
than 1/5 times the peak area of mitiglinide obtained from
the standard solution, and the total area of peaks other than
mitiglinide from sample solution is not larger than 3/10
times the peak area of mitiglinide from the standard solu-
tion.

Operating conditions—

Detector, column, and column temperature: Proceed as
directed in the operating conditions in the Assay.

Mobile phase: Adjust to pH 2.0 of a mixture of water,
acetonitrile for liquid chromatography and *n*-amyl alcohol
(66:33:1) with phosphoric acid.

Flow rate: Adjust so that the retention time of mitiglinide
is about 12 minutes.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard
solution, add a mixture of water and acetonitrile (2:1) to
make exactly 50 mL. Confirm that the peak area of
mitiglinide obtained with 15 µL of this solution is equivalent
to 7 to 13 % of that obtained with 15 µL of the standard so-
lution.

System performance: When the procedure is run with 15
µL of the standard solution under the above operating con-
ditions, the number of theoretical plates and the symmetry
factor of the peak of mitiglinide are not less than 4000 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 operat-
ing conditions, the relative standard deviation of the peak
area of mitiglinide is not more than 2.0%.

Water <2.48>   4.5 – 6.0% (50 mg, coulometric titration).

Assay   Weigh accurately about 50 mg each of Mitiglinide
Calcium Hydrate and Mitiglinide Calcium RS (separately de-
termine the water <2.48> in the same manner as Mitiglinide
Calcium Hydrate), add a mixture of water and acetonitrile
(2:1) to them, dissolve by sonicating while occasional shak-
ing, and add the mixture of water and acetonitrile (2:1) to
make exactly 50 mL. Pipet 10 mL each of these solutions,
add exactly 10 mL of the internal standard solution, add the
mixture of water and acetonitrile (2:1) to make 100 mL, and
use these solutions as the sample solution and the standard
solution, respectively. Perform the test with 10 µL of the
sample solution and standard solution as directed under Liq-
uid Chromatography <2.01> according to the following con-
ditions, and calculate the ratios, Q_T and Q_S, of the peak area

of mitiglinide to that of the internal standard.

Amount (mg) of mitiglinide calcium hydrate
$$(C_{38}H_{48}CaN_2O_6.2H_2O) = M_S \times Q_T/Q_S \times 1.054$$

M_S: Amount (mg) of Mitiglinide Calcium RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of 2-nitrophenol in acetonitrile (1 in 5000).
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 palmitamide propylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: Adjust to pH 2.0 of a mixture of water, acetonitrile for liquid chromatography and *n*-amyl alcohol (62:37:1) with phosphoric acid.
Flow rate: Adjust so that the retention time of mitiglinide is about 7.5 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 mitiglinide 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 mitiglinide is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Mitiglinide Calcium Tablets

ミチグリニドカルシウム錠

Mitiglinide Calcium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mitiglinide calcium hydrate ($C_{38}H_{48}CaN_2O_6.2H_2O$: 704.91).

Method of preparation Prepare as directed under Tablets, with Mitiglinide Calcium Hydrate.

Identification To 5 mL of the sample solution obtained in the Purity, add a mixture of water and acetonitrile (2:1) to make 10 mL, and use this solution as the sample solution. Separately, to 50 mg of mitiglinide calcium hydrate add the mixture of water and acetonitrile (2:1), dissolve by sonicating while occasional shaking, add the mixture of water and acetonitrile (2:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the principal peaks in the chromatograms obtained from the sample solution and standard solution show the same retention time, and both spectra of these peaks exhibit similar intensities of absorption at the same wavelengths.
Operating conditions—
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the

Purity.
Detector: Photodiode array detector (wavelength: 210 nm, spectrum range of measurement: 200 – 360 nm).
System suitability—
System performance: Proceed as directed in the system suitability in the Purity.

Purity Related substances—Take not less than 10 tablets of Mitiglinide Calcium Tablets, and powder. Weigh a portion of the powder, equivalent to 50 mg of Mitiglinide Calcium Hydrate, add 35 mL of a mixture of water and acetonitrile (2:1), sonicate whiles occasional shaking, add the mixture of water and acetonitrile (2:1) to make 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mixture of water and acetonitrile (2:1) 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. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.2 to mitiglinide, obtained from the sample solution is not larger than 1/4 times the peak area of mitiglinide obtained from the standard solution, and the area of peak other than mitiglinide and the peak mentioned above from the sample solution is not larger than 1/8 times the peak area of mitiglinide from the standard solution. In addition, the total area of the peaks other than mitiglinide from the sample solution is not larger than 1/2 times the peak area of mitiglinide 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 palmitamide propylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: Adjust to pH 2.0 of a mixture of water, acetonitrile for liquid chromatography and *n*-amyl alcohol (66:33:1) with phosphoric acid.
Flow rate: Adjust so that the retention time of mitiglinide is about 12 minutes.
Time span of measurement: About 2 times as long as the retention time of mitiglinide, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 2.5 mL of the standard solution, add the mixture of water and acetonitrile (2:1) to make exactly 50 mL. Confirm that the peak area of mitiglinide obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 15 μL of the standard solution.
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 mitiglinide are not less than 3000 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 mitiglinide is not more than 1.5%.

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 Mitiglinide Calcium Tablets add a mixture of water and acetonitrile (2:1), add exactly $V/10$ mL of the internal standard solution, sonicate while occasional shaking, then add the mixture of water and acetonitrile (2:1) to make V mL so that each mL contains about 0.1 mg of mitiglinide calcium hydrate ($C_{38}H_{48}CaN_2O_6.2H_2O$), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Mitiglinide Calcium RS (separately determine the water <2.48> in the same manner as Mitiglinide Calcium Hydrate), add the mixture of water and acetonitrile (2:1), dissolve by sonicating while occasional shaking, and add the mixture of water and acetonitrile (2:1) to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (2:1) to make 100 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 mitiglinide to that of the internal standard.

$$\text{Amount (mg) of mitiglinide calcium hydrate}$$
$$(C_{38}H_{48}CaN_2O_6.2H_2O)$$
$$= M_S \times Q_T/Q_S \times V/500 \times 1.054$$

M_S: Amount (mg) of Mitiglinide Calcium RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of 2-nitrophenol in acetonitrile (1 in 5000).
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Mitiglinide Calcium Hydrate.
System suitability—
Proceed as directed in the operating conditions in the Assay.

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 Mitiglinide Calcium Tablets is not less than 85%.

Start the test with 1 tablet of Mitiglinide Calcium Tablets, 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 1 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mixture of water and acetonitrile (2:1) to make exactly V' mL so that each mL contains about 5.6 μg of mitiglinide calcium hydrate ($C_{38}H_{48}CaN_2O_6.2H_2O$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mitiglinide Calcium RS (separately determine the water <2.48> in the same manner as Mitiglinide Calcium Hydrate), add the mixture of water and acetonitrile (2:1), dissolve by treating with ultrasonic waves while occasional shaking, and add the mixture of water and acetonitrile (2:1) 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 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 mitiglinide in each solution.

Dissolution rate (%) with respect to the labeled amount of mitiglinide calcium hydrate ($C_{38}H_{48}CaN_2O_6.2H_2O$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18 \times 1.054$$

M_S: Amount (mg) of Mitiglinide Calcium RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of mitiglinide calcium hydrate ($C_{38}H_{48}CaN_2O_6.2H_2O$) in 1 tablet.

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Mitiglinide Calcium Hydrate.
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 mitiglinide 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 mitiglinide is not more than 1.5%.

Assay   Weigh accurately the mass of not less than 20 tablets of Mitiglinide Calcium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mitiglinide calcium hydrate ($C_{38}H_{48}CaN_2O_6.2H_2O$), add a mixture of water and acetonitrile (2:1), add exactly 10 mL of the internal standard solution, sonicate while occasional shaking, then add the mixture of water and acetonitrile (2:1) to make 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Mitiglinide Calcium RS (separately determine the water <2.48> in the same manner as Mitiglinide Calcium Hydrate), add the mixture of water and acetonitrile (2:1), dissolve by sonicating while occasional shaking, and add the mixture of water and acetonitrile (2:1) to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (2:1) to make 100 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 mitiglinide to that of the internal standard.

$$\text{Amount (mg) of mitiglinide calcium hydrate}$$
$$(C_{38}H_{48}CaN_2O_6.2H_2O)$$
$$= M_S \times Q_T/Q_S \times 1/5 \times 1.054$$

M_S: Amount (mg) of Mitiglinide Calcium RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of 2-nitrophenol in acetonitrile (1 in 5000).
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Mitiglinide Calcium Hydrate.
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 mitiglinide are eluted in this order with the resolution between these peaks being not less than 10.
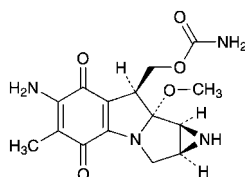
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak of mitiglinide to that of the internal standard is not

more than 1.0%.

Containers and storage　Containers—Well-closed containers.

Mitomycin C

マイトマイシン C

C$_{15}$H$_{18}$N$_4$O$_5$: 334.33
(1a*S*,8*S*,8a*R*,8b*S*)-6-Amino-4,7-dioxo-8a-methoxy-
5-methyl-1,1a,2,8,8a,8b-
hexahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-
8-ylmethyl carbamate
[*50-07-7*]

　Mitomycin C is a substance having antitumor activity produced by the growth of *Streptomyces caespitosus*.

　It contains not less than 970 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the dried basis. The potency of Mitomycin C is expressed as mass (potency) of mitomycin C (C$_{15}$H$_{18}$N$_4$O$_5$).

Description　Mitomycin C occurs as blue-purple, crystals or crystalline powder.

　It is freely soluble in *N*,*N*-dimethylacetamide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

Identification　**(1)**　Determine the absorption spectrum of a solution of Mitomycin C (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 Mitomycin C 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 Mitomycin C 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 Mitomycin C RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity　Related substances—Conduct this procedure rapidly after the sample and the standard solutions are prepared. Dissolve 50 mg of Mitomycin C 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 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 area of the peak other than mitomycin C obtained from the sample solution is not larger than the peak area of mitomycin C obtained from the standard solution, and the total area of the peaks other than mitomycin C from the sample solution is not larger than 3 times the peak area of mitomycin C from the standard solution.

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 30°C.
　Mobile phase A: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.
　Mobile phase B: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.
　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 → 0	0 → 100
30 – 45	0	100

　Flow rate: About 1.0 mL per minute.
　Time span of measurement: About 2 times as long as the retention time of mitomycin C, beginning after the solvent peak.
System suitability—
　Test for required detectability: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C 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 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.
　System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0%.

Loss on drying <2.41>　Not more than 1.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay　Weigh accurately an amount of Mitomycin C and Mitomycin C RS, equivalent to about 25 mg (potency), dissolve each in *N*,*N*-dimethylacetamide 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 mitomycin C in each solution.

　Amount [μg (potency)] of mitomycin C (C$_{15}$H$_{18}$N$_4$O$_5$)
　　= $M_S × A_T/A_S × 1000$

M_S: Amount [mg (potency)] of Mitomycin C RS taken

Operating conditions—
　Detector: An ultraviolet absorption photometer (wavelength: 365 nm).
　Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with phenylated silica gel for

liquid chromatography (10 μm in particle diameter).

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

Mobile phase: To 40 mL of 0.5 mol/L ammonium acetate TS add 5 mL of diluted acetic acid (100) (1 in 20) and water to make 1000 mL. To 600 mL of this solution add 200 mL of methanol.

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

System suitability—

System performance: Dissolve about 25 mg of Mitomycin C RS and about 0.375 g of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of *N*,*N*-dimethylacetamide. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde 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 mitomycin C is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Mitomycin C for Injection

注射用マイトマイシン **C**

Mitomycin C 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 mitomycin C ($C_{15}H_{18}N_4O_5$: 334.33).

Method of preparation   Prepare as directed under Injections, with Mitomycin C.

Description   Mitomycin C for Injection occurs as a blue-purple powder.

Identification   Dissolve an amount of Mitomycin C for Injection, equivalent to 2 mg (potency) of Mitomycin C, in 200 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 216 nm and 220 nm, and between 362 nm and 366 nm.

pH <2.54>   The pH of a solution, prepared by dissolving 0.25 g of Mitomycin C for Injection in 20 mL of water, is 5.5 to 8.5.

Loss on drying <2.41>   Not more than 1.0% (0.4 g, in vacuum not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins <4.01>   Less than 10 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 the content of 1 container of Mitomycin C for Injection add exactly *V* mL of *N*,*N*-dimethylacetamide so that each mL contains about 0.5 mg (potency) of Mitomycin C, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg (potency) of Mitomycin C RS, add *N*,*N*-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

Amount [mg (potency)] of mitomycin C ($C_{15}H_{18}N_4O_5$)
$$= M_S \times A_T/A_S \times V/50$$

M_S: Amount [mg (potency)] of Mitomycin C RS taken

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 Mitomycin C for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Mitomycin C, add exactly 20 mL of *N*,*N*-dimethylacetamide, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount of Mitomycin C RS, equivalent to about 25 mg (potency), dissolve in *N*,*N*-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.
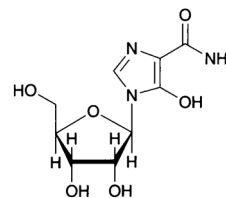
Amount [mg (potency)] of mitomycin C ($C_{15}H_{18}N_4O_5$)
$$= M_S \times A_T/A_S \times 2/5$$

M_S: Amount [mg (potency)] of Mitomycin C RS taken

Containers and storage   Containers—Hermetic containers.

Mizoribine

ミゾリビン

$C_9H_{13}N_3O_6$: 259.22
5-Hydroxy-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamide
[*50924-49-7*]

Mizoribine contains not less than 98.0% and not more than 102.0% of mizoribine ($C_9H_{13}N_3O_6$), calculated on the anhydrous basis.

Description   Mizoribine occurs as a white to yellowish white crystalline powder.

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

Identification   (1)   Determine the absorption spectrum of a solution of Mizoribine (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 Mizoribine 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 Mizoribine 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 Mizoribine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-25 - -27°$ (0.5 g calculated

on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mizoribine 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) Related substances—Dissolve 0.10 g of Mizoribine in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 5 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 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 areas of the peaks other than mizoribine obtained from the sample solution are not larger than the peak area of mizoribine obtained 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 mizoribine, 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 5 mL. Confirm that the peak area of mizoribine obtained from 5 μL of this solution is equivalent to 14 to 26% 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, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, 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 mizoribine is not more than 2.0%.

Water <*2.48*>   Not more than 0.5% (0.5 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.1 g of Mizoribine, 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 sample solution. Separately, weigh accurately about 10 mg of Mizoribine RS (separately determine the water <*2.48*> using the same manner as Mizoribine), dissolve in the mobile phase to make exactly 50 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 mizoribine in each solution.

$$\text{Amount (mg) of mizoribine } (C_9H_{13}N_3O_6)$$
$$= M_S \times A_T/A_S \times 10$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-length: 279 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: Diluted phosphoric acid (1 in 1500).

Flow rate: Adjust so that the retention time of mizoribine 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 number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, 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 mizoribine is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—At a temperature between 2 and 8°C.

Mizoribine Tablets

ミゾリビン錠

Mizoribine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of mizoribine ($C_9H_{13}N_3O_6$: 259.22).

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

Identification To a quantity of powdered Mizoribine Tablets, equivalent to 0.1 g of Mizoribine, add 5 mL of water, shake, filter, and use the filtrate as the sample solution. Separately, dissolve 20 mg of Mizoribine RS in 1 mL of water, and use this solution as the standard solution. Perform the test with the sample solution and standard solution 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. Then develop the plate with a mixture of methanol, ammonia solution (28) and 1-propanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the principal spot from the sample solution and the spot from the standard solution show a red-brown color and the same Rf value.

Purity Related substances—To a quantity of powdered Mizoribine Tablets, equivalent to 0.10 g of Mizoribine, add 30 mL of the mobile phase, shake, then add the mobile phase to make 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.5 μm and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Pipet 1 mL of the solution, add the mobile phase to make exactly 20 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 by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 to mizoribine, obtained from the sample solution is not larger than the peak area of mizoribine obtained from the standard solution, and the area of the peak other than mizoribine and

the peak mentioned above is not larger than 2/5 times the peak area of mizoribine from the standard solution.

Operating conditions—

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

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

Time span of measurement: About 3 times as long as the retention time of mizoribine, 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 5 mL. Confirm that the peak area of mizoribine obtained from 5 μL of this solution is equivalent to 14 to 26% 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, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, 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 mizoribine 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 Mizoribine Tablets add 50 mL of water, shake until the tablet is disintegrated, and add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5 μg of mizoribine ($C_9H_{13}N_3O_6$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount of mizoribine } (C_9H_{13}N_3O_6)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/50$$

M_S: Amount (mg) of Mizoribine 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 Mizoribine Tablets is not less than 80%.

Start the test with 1 tablet of Mizoribine 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 not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 14 μg of mizoribine ($C_9H_{13}N_3O_6$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Mizoribine RS (separately determine the water *<2.48>* in the same manner as Mizoribine), and dissolve in 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. Determine the absorbances, A_T and A_S, at 279 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 mizoribine ($C_9H_{13}N_3O_6$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

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

C: Labeled amount (mg) of mizoribine ($C_9H_{13}N_3O_6$) in 1 tablet

Assay   Weigh accurately not less than 20 Mizoribine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of mizoribine ($C_9H_{13}N_3O_6$), add 50 mL of water and shake, then add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mizoribine RS (separately determine the water *<2.48>* in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 2 mL of the 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 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.
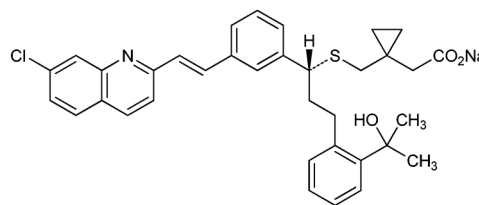
$$\text{Amount (mg) of mizoribine } (C_9H_{13}N_3O_6) = M_S \times A_T/A_S$$

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

Containers and storage   Containers—Tight containers.

Montelukast Sodium

モンテルカストナトリウム

$C_{35}H_{35}ClNNaO_3S$: 608.17
Monosodium (1-{[((1R)-1-{3-[(1E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl}cyclopropyl)acetate
[*151767-02-1*]

Montelukast Sodium contains not less than 98.0% and not more than 102.0% of montelukast sodium ($C_{35}H_{35}ClNNaO_3S$), calculated on the anhydrous and residual solvent-free basis.

Description   Montelukast Sodium occurs as a white to pale yellow-white powder.

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

It is hygroscopic.

It turns yellow on exposure to light.

It shows crystal polymorphism.

Identification   **(1)**   Place 0.1 g of Montelukast Sodium in a crucible, and ignite until a white residue is formed. To the residue add 2 mL of water, and then filter. To the filtrate add 2 mL of potassium carbonate solution (3 in 20), and heat to boiling: no precipitate is observed. To this solution add 4 mL of potassium hexahydroxoantimonate (V) TS, heat to boiling, and cool immediately in ice water: a white precipitate is formed. Rub the inside wall of the test tube with a

glass rod, if necessary.

(2) Determine the absorption spectrum of a solution of Montelukast Sodium in a mixture of methanol and water (3:1) (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 Montelukast Sodium RS for Identification 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 Montelukast Sodium as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Montelukast Sodium RS for Identification: both spectra exhibit similar intensities of absorption at the same wave numbers. Or, perform the test by the potassium bromide disk method or the ATR method, and compare the spectrum with the spectrum of Montelukast Sodium RS for Identification: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Montelukast Sodium and Montelukast Sodium RS for Identification in toluene, add heptane, shake, then allow to stand, and remove the supernatant liquid by decantation. Dry the residue at 75°C for 16 hours under reduced pressure, and perform the test by paste method, potassium bromide disk method or the ATR method.

Purity (1) Heavy metals—Dissolve 0.5 g of Montelukast Sodium in 20 mL of a mixture of acetone and water (4:1), and use this solution as the sample solution. Separately, take 0.5 mL of Standard Lead Solution, add 20 mL of the mixture of acetone and water (4:1), and use this solution as the standard solution. To the sample solution and the standard solution add 2 mL of acetate buffer solution (pH 3.5), and shake. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, shake immediately, then allow to stand for 2 minutes, and filter through a membrane filter with a pore size 0.45 μm (about 13 mm in diameter). Compare the color on the membrane filters through which each solution is filtered: the color obtained from the sample solution is not darker than that obtained from the standard solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Montelukast Sodium in 50 mL of a mixture of methanol and water (9:1), 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 the amount of them by the area percentage method: the amount of the peak of related substance A, having the relative retention time of about 0.4 to montelukast is not more than 0.2%, the amounts of the peaks, related substance B and related substance E, having respectively the relative retention times of about 0.8 and about 1.2 are not more than 0.15%, the total amount of the two peaks, related substances C and D, both having the relative retention time about 0.9 is not more than 0.15%, the amount of the peak of related substance F having the relative retention time of about 1.9 is not more than 0.3%, and the amount of the peak other than montelukast and other than the peaks mentioned above is not more than 0.10%. Furthermore, the total amount of the peaks other than montelukast is not more than 0.6%.

Operating conditions—

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

Time span of measurement: For 16 minutes after injection, 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 sample solution, add the mixture of methanol and water (9:1) to make exactly 100 mL. Pipet 1 mL of this solution, add the mixture of methanol and water (9:1) to make exactly 20 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the SN ratio of the peak of montelukast is not less than 10.

For the calculations mentioned above, the peak areas smaller than that of montelukast, founded in the chromatogram obtained with 10 μL of the solution for system suitability test, are excluded.

(3) Optical isomer—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Montelukast Sodium in 50 mL of a mixture of water and acetonitrile (1:1), 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 the amounts of them by the area percentage method: the amount of the peak having the relative retention time of about 0.7 to montelukast is not more than 0.2%.

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 α_1-acid glycoprotein binding silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase A: Dissolve 2.3 g of ammonium acetate in 1000 mL of water, and adjust to pH 5.7 with acetic acid (100).

Mobile phase B: A mixture of methanol and acetonitrile (3:2).

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 – 30	70 → 60	30 → 40
30 – 35	60	40

Flow rate: 0.9 mL per minute (the retention time of montelukast is about 25 minutes).

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (1:1) to make exactly 100 mL. Pipet 1 mL of this solution, add the mixture of water and acetonitrile (1:1) to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the SN ratio of the peak of montelukast is not less than 10.

System performance: When the procedure is run with 10 μL of a solution of Montelukast Racemate RS for System Suitability in the mixture of water and acetonitrile (1:1) (1 in 10,000) under the above operating conditions, the resolution

between the peak of montelukast and the peak having the relative retention time of about 0.7 to montelukast is not less than 2.9.

Water <2.48>   Not more than 4.0% (0.3 g, volumetric titration, direct titration).

Assay   Conduct this procedure using light-resistant vessels. Weigh accurately about 50 mg of Montelukast Sodium, and dissolve in a mixture of methanol and water (9:1) to make exactly 50 mL. Pipet 10 mL of this solution, add the mixture of methanol and water (9:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 26 mg of Montelukast Dicyclohexylamine RS, dissolve in the mixture of methanol and water (9:1) to make exactly 50 mL. Pipet 5 mL of this solution, add the mixture of methanol and water (9:1) 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 the peak areas, A_T and A_S, of montelukast in each solution.

Amount (mg) of montelukast sodium ($C_{35}H_{35}ClNNaO_3S$)
$$= M_S \times A_T/A_S \times 5/2 \times 0.792$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

Operating conditions—
  Detector: An ultraviolet absorption photometer (wavelength: 238 nm).
  Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with phenylsilanized silica gel for liquid chromatography (1.8 μm in particle diameter).
  Column temperature: A constant temperature of about 30°C.
  Mobile phase A: A mixture of water and trifluoroacetic acid (2000:3).
  Mobile phase B: A mixture of acetonitrile and trifluoroacetic acid (2000:3).
  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 – 3	60	40
3 – 16	60 → 49	40 → 51

  Flow rate: 1.2 mL per minute (the retention time of montelukast is about 7 minutes).
System suitability—
  System performance: Use a solution of Montelukast RS for System Suitability in the mixture of methanol and water (9:1) (1 in 1000) as the solution A for peak identification. Perform the test with 10 μL of the solution A for peak identification under the above operating conditions, and identify the peaks having the relative retention times to montelukast of about 0.4 (related substance A), about 0.9 (related substances C and D), about 1.2 (related substance E), and about 1.9 (related substance F). Place 1 mL of the solution A for peak identification in a clear glass container, allow to stand for about 20 minutes, and use this solution as the solution B for peak identification. When the procedure is run with 10 μL of the solution B for peak identification under the above operating conditions, and identify the peak having the relative retention time of about 0.8 to montelukast (related substance B), the resolution between the peaks of related sub-

stance B and montelukast is not less than 2.5, and between the peaks of montelukast and related substance E is not less than 1.5.
  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 montelukast is not more than 0.73%.
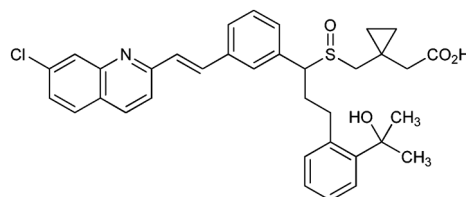
Containers and storage   Containers—Tight containers
    Storage—Light-resistant.
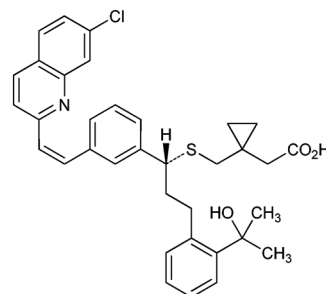
Others
Related substance A:
(1-{[(1-{3-[(1*E*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfinyl]methyl}cyclopropyl)acetic acid
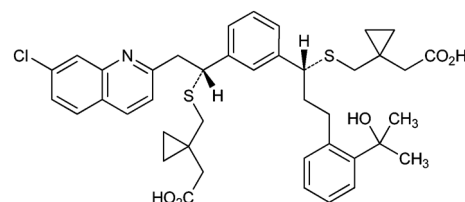

Related substance B:
(1-{[((1*R*)-1-{3-[(1*Z*)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl}cyclopropyl)acetic acid
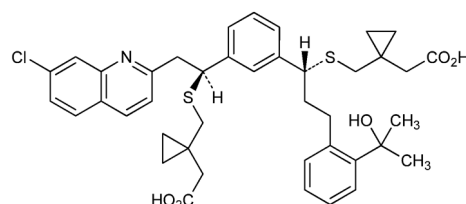

Related substance C:
(1-{[((1*R*)-1-{3-[(1*R*)-1-({[1-(Carboxymethyl)cyclopropyl]methyl}sulfanyl)-2-(7-chloroquinolin-2-yl)ethyl]phenyl}-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl}cyclopropyl)acetic acid
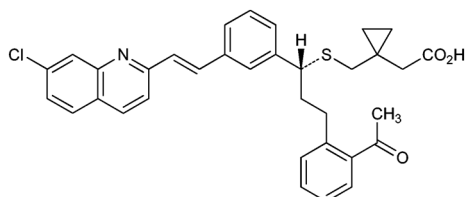

Related substance D:
(1-{[((1*R*)-1-{3-[(1*S*)-1-({[1-(Carboxymethyl)cyclopropyl]methyl}sulfanyl)-2-(7-chloroquinolin-2-yl)ethyl]phenyl}-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl)sulfanyl]methyl}cyclopropyl)acetic acid
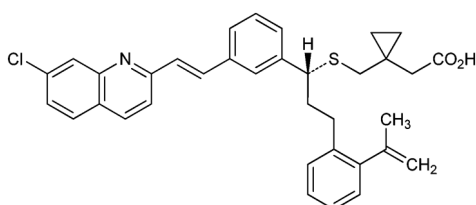
Related substance E:

(1-{[((1R)-3-(2-Acetylphenyl)-1-{3-[(1E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}propyl)sulfanyl]methyl}cyclopropyl)acetic acid

Related substance F:

(1-{[((1R)-1-{3-[(1E)-2-(7-Chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(1-methylethenyl)phenyl]propyl)sulfanyl]methyl}cyclopropyl)acetic acid

Montelukast Sodium Chewable Tablets

モンテルカストナトリウムチュアブル錠

Montelukast Sodium Chewable Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$: 586.18).

Method of preparation   Prepare as directed under Chewable Tablets, with Montelukast Sodium.

Identification   To an amount of powdered Montelukast Sodium Chewable Tablets, equivalent to 5 mg of montelukast ($C_{35}H_{36}ClNO_3S$), add 500 mL of a mixture of methanol and water (3:1), shake, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 281 nm and 285 nm, between 325 nm and 329 nm, between 343 nm and 347 nm and between 357 nm and 361 nm.

Purity   Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and water (3:1) 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 two peaks of related substance A, having the relative retention time of about 0.45 to montelukast, obtained from the sample solution is not larger than 1.5 times the peak area of montelukast obtained from the standard solution, the area of related substance B having the relative retention time of about 0.92 to montelukast, obtained from the sample solution is not larger than 3/20 times the peak area of montelukast obtained from the standard solution, and the area of the peaks other than montelukast and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of montelukast from the standard solution. Furthermore, the total area of the peaks other than montelukast is not larger than 1.8 times the peak area of montelukast from the standard solution. However, the peaks of the related substances derived from Montelukast Sodium [having the relative retention time of about 1.04 (related substance E), about 1.16 (related substance C), about 1.18 (related substance D), about 1.24 and about 1.55 (related substance F) to montelukast] are excluded. For the area of the peak, having the relative retention time of about 0.71 to montelukast, 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 1.5 times as long as the retention time of montelukast, beginning after the solvent peak.

System suitability—

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

Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of methanol and water (3:1) to make exactly 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the SN ratio of the peak of montelukast is not less than 10.

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 peak area of montelukast 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.

Conduct this procedure using light-resistant vessels. To 1 tablet of Montelukast Sodium Chewable Tablets add 50 mL of water to disintegrate the tablet, add a suitable amount of methanol, and disperse the fine particles by sonicating. Add methanol to make exactly 200 mL, and centrifuge or filter. Pipet *V* mL of this solution, add a mixture of methanol and water (3:1) to make exactly *V'* mL so that each mL contains about 25 μg of montelukast ($C_{35}H_{36}ClNO_3S$) and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Montelukast Dicyclohexylamine RS, and dissolve in a mixture of methanol and water (3:1) to make exactly 200 mL. Pipet 20 mL of this solution, add a mixture of methanol and water (3:1) 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 montelukast in each solution.

$$\text{Amount (mg) of montelukast } (C_{35}H_{36}ClNO_3S)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/5 \times 0.764$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 389 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 10 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A solution of trifluoroacetic acid in a mixture of water and acetonitrile for liquid chromatography (1:1) (1 in 500).

Flow rate: Adjust so that the retention time of montelukast is about 2 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 montelukast are not less than 2000 and not more than 1.5, respectively.
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 montelukast 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 a solution of sodium lauryl sulfate (1 in 200) as the dissolution medium, the dissolution rate in 20 minutes of Montelukast Sodium Chewable Tablets is not less than 85%.
Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Montelukast Sodium Chewable Tablets, withdraw not less than 15 mL of the medium at the specified minute after starting the test, and centrifuge. Pipet V mL of the supernatant liquid, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of montelukast ($C_{35}H_{36}ClNO_3S$), and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Montelukast Dicyclohexylamine RS, dissolve in methanol to make exactly 100 mL. Pipet 2 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 montelukast in each solution.

Dissolution rate (%) with respect to the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18 \times 0.764$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken
C: Labeled amount (mg) of montelukast ($C_{35}H_{36}ClNO_3S$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Uniformity of dosage units.
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 montelukast are not less than 2000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 5 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of montelukast is not more than 2.0%.

Assay   Conduct this procedure using light-resistant vessels. Disintegrate 10 tablets of Montelukast Sodium Chewable Tablets in 150 mL of a mixture of methanol and water (3:1), disperse the fine particles by sonicating, and add a mixture of methanol and water (3:1) to make exactly 200 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of methanol and water (3:1) to make exactly V' mL so that each mL contains about 0.25 mg of montelukast ($C_{35}H_{36}ClNO_3S$), and use this solution as the sample solution. Separately, weigh accurately

about 33 mg of Montelukast Dicyclohexylamine RS, and dissolve in a mixture of methanol and water (3:1) 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 montelukast in each solution.

Amount (mg) of montelukast ($C_{35}H_{36}ClNO_3S$) in 1 tablet
$$= M_S \times A_T/A_S \times V'/V \times 1/5 \times 0.764$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 255 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with phenylhexylsilanized silica gel for liquid chromatography (3 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase A: A solution of trifluoroacetic acid (1 in 500).
Mobile phase B: A mixture of methanol and acetonitrile for liquid chromatography (3:2).
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	48 → 45	52 → 55
5 – 12	45	55
12 – 22	45 → 25	55 → 75
22 – 23	25	75

Flow rate: 1.5 mL per minute (the retention time of montelukast is about 14 minutes).
System suitability—
System performance: Take 10 mL of the standard solution in a transparent vessel, add 4 μL of hydrogen peroxide (30), and allow to stand under 4000 lx white light for 10 minutes. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peak of related substance B, having a relative retention time of about 0.92 to montelukast and the peak of montelukast is not less than 1.5. And proceed with 20 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of montelukast are not less than 5000 and not more than 2.5, respectively.
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 peak area of montelukast is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Storage—Light-resistant.

Others
Related substances A, B, C, D, E and F: Refer to them described in Montelukast Sodium.

Montelukast Sodium Tablets

モンテルカストナトリウム錠

Montelukast Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$: 586.18).

Method of preparation Prepare as directed under Tablets, with Montelukast Sodium.

Identification To an amount of powdered Montelukast Sodium Tablets, equivalent to 5 mg of montelukast ($C_{35}H_{36}ClNO_3S$), add 500 mL of a mixture of methanol and water (3:1), shake, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 281 nm and 285 nm, between 325 nm and 329 nm, between 343 nm and 347 nm and between 357 nm and 361 nm.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and water (3:1) 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 two peaks of related substance A, having the relative retention time of about 0.45 to montelukast, obtained from the sample solution is not larger than the peak area of montelukast obtained from the standard solution, the area of related substance B having the relative retention time of about 0.92 to montelukast, obtained from the sample solution is not larger than 3/20 times the peak area of montelukast obtained from the standard solution, and the area of the peaks other than montelukast and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of montelukast from the standard solution. Furthermore, the total area of the peaks other than montelukast from the sample solution is not larger than 1.2 times the peak area of montelukast from the standard solution. However, the peaks of the related substances derived from Montelukast Sodium [having the relative retention time of about 1.04 (related substance E), about 1.16 (related substance C), about 1.18 (related substance D), about 1.24 and about 1.55 (related substance F) to montelukast] are excluded. For the area of the peak, having the relative retention time of about 0.71 to montelukast, 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 1.5 times as long as the retention time of montelukast, beginning after the solvent peak.
System suitability—
System performance: Proceed as directed in the system suitability in the Assay.
Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of methanol and water (3:1) to make exactly 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the SN ratio of the peak of montelukast is not less than 10.
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of montelukast 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.
Conduct this procedure using light-resistant vessels. To 1 tablet of Montelukast Sodium Tablets add 50 mL of water to disintegrate the tablet, add a suitable amount of methanol, and disperse the fine particles by sonicating. Add methanol to make exactly 200 mL, and centrifuge or filter. Pipet V mL of this solution, add a mixture of methanol and water (3:1) to make exactly V' mL so that each mL contains about 25 μg of montelukast ($C_{35}H_{36}ClNO_3S$) and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Montelukast Dicyclohexylamine RS, and dissolve in a mixture of methanol and water (3:1) to make exactly 200 mL. Pipet 20 mL of this solution, add a mixture of methanol and water (3:1) 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 montelukast in each solution.

$$\text{Amount (mg) of montelukast } (C_{35}H_{36}ClNO_3S)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/5 \times 0.764$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 389 nm).
Column: A stainless steel column 3.0 mm in inside diameter and 10 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 50°C.
Mobile phase: A solution of trifluoroacetic acid in a mixture of water and acetonitrile for liquid chromatography (1:1) (1 in 500).
Flow rate: Adjust so that the retention time of montelukast is about 2 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 montelukast are not less than 2000 and not more than 1.5, respectively.
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 montelukast 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 a solution of sodium lauryl sulfate (1 in 200) as the dissolution medium, the dissolution rate in 20 minutes of Montelukast Sodium Tablets is not less than 85%.
Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Montelukast Sodium Tablets, withdraw not less than 15 mL of the medium at the specified minute after starting the test, and centrifuge. Pipet V mL of the supernatant liquid, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of montelukast ($C_{35}H_{36}ClNO_3S$), and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Montelukast Dicyclohexylamine RS, and dissolve in

methanol to make exactly 100 mL. Pipet 2 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 montelukast in each solution.

Dissolution rate (%) with respect to the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18 \times 0.764$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

C: Labeled amount (mg) of montelukast ($C_{35}H_{36}ClNO_3S$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Uniformity of dosage units.

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 montelukast are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of montelukast is not more than 2.0%.

Assay   Conduct this procedure using light-resistant vessels. Disintegrate 10 tablets of Montelukast Sodium Tablets in 150 mL of a mixture of methanol and water (3:1), disperse the fine particles by sonicating, and add a mixture of methanol and water (3:1) to make exactly 200 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of methanol and water (3:1) to make exactly V' mL so that each mL contains about 0.25 mg of montelukast ($C_{35}H_{36}ClNO_3S$), and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Montelukast Dicyclohexylamine RS, and dissolve in a mixture of methanol and water (3:1) 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 montelukast in each solution.

Amount (mg) of montelukast ($C_{35}H_{36}ClNO_3S$) in 1 tablet

$$= M_S \times A_T/A_S \times V/V' \times 1/5 \times 0.764$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with phenylhexylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase A: A solution of trifluoroacetic acid (1 in 500).

Mobile phase B: A mixture of methanol and acetonitrile for liquid chromatography (3:2).

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	48 → 45	52 → 55
5 – 12	45	55
12 – 22	45 → 25	55 → 75
22 – 23	25	75

Flow rate: 1.5 mL per minute (the retention time of montelukast is about 14 minutes).

System suitability—

System performance: Take 10 mL of the standard solution in a transparent vessel, add 4 μL of hydrogen peroxide (30), and allow to stand under 4000 lx white light for 10 minutes. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peak of related substance B, having a relative retention time of about 0.92 to montelukast and the peak of montelukast is not less than 1.5. And proceed with 20 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of montelukast are not less than 5000 and not more than 2.5, respectively.

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 peak area of montelukast is not more than 1.0%.

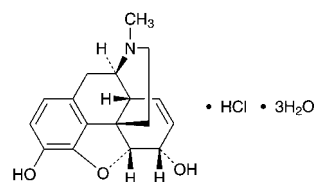**Containers and storage**   Containers—Tight containers.
Storage—Light-resistant.

Others
Related substances A, B, C, D, E and F: Refer to them described in Montelukast Sodium.

Morphine Hydrochloride Hydrate

モルヒネ塩酸塩水和物

$C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$: 375.84
(5R,6S)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol monohydrochloride trihydrate
[*6055-06-7*]

Morphine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of morphine hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl$: 321.80), calculated on the anhydrous basis.

Description   Morphine Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

It gradually becomes yellow-brown by light.

Identification   (1)   Determine the absorption spectrum of a solution of Morphine Hydrochloride Hydrate (1 in 10,000)

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 Morphine Hydrochloride Hydrate in dilute sodium hydroxide TS (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.

(2) Determine the infrared absorption spectrum of Morphine 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 Morphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-111 - -116°$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.40 g of Morphine Hydrochloride Hydrate in 10 mL of water: the solution is clear. When perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, the absorbance at 420 nm is not more than 0.12.

(2) Sulfate <1.14>—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

(3) Meconic acid—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(4) Related substances—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 10 mL of diluted methanol (4 in 5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add diluted methanol (4 in 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. Develop the plate with a mixture of acetone, ethanol (99.5) and ammonia solution (28) (21:14:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot having a Rf value of about 0.17 obtained with the sample solution is not more intense than the spot obtained with the standard solution (1), and the spots other than the principal spot, the spot mentioned above and the spot of the starting point are not more intense than the spot with the standard solution (2).

Water <2.48> 13 – 15% (0.1 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 Morphine Hydrochloride Hydrate, dissolve in 3.0 mL of formic acid, add 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), mix, 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
= 32.18 mg of $C_{17}H_{19}NO_3.HCl$

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

Morphine Hydrochloride Injection

モルヒネ塩酸塩注射液

Morphine Hydrochloride Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84).

Method of preparation Prepare as directed under Injections, with Morphine Hydrochloride Hydrate.

Description Morphine Hydrochloride Injection is a clear, colorless or pale yellow-brown liquid.

It gradually becomes yellow-brown by light.

pH: 2.5 – 5.0

Identification Take a volume of Morphine Hydrochloride Injection, equivalent to 0.04 g of Morphine Hydrochloride Hydrate, add water to make 20 mL, and use this solution as the sample solution. To 5 mL of the sample solution add water to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm. And to 5 mL of the sample solution add dilute sodium hydroxide TS to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 296 nm and 300 nm.

Bacterial endotoxins <4.01> Less than 1.5 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 Take exactly a volume of Morphine Hydrochloride Injection, equivalent to about 80 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), and add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride hydrate for assay, dissolve in exactly 10 mL of the internal standard solution, add water 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 morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride hydrate
($C_{17}H_{19}NO_3.HCl.3H_2O$)
= $M_S \times Q_T/Q_S \times 4 \times 1.168$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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, and mix.

Flow rate: Adjust so that retention time of morphine 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, morphine 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 ratios of the peak area of morphine 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.

Morphine Hydrochloride Tablets

モルヒネ塩酸塩錠

Morphine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$.HCl.3H$_2$O: 375.84).

Method of preparation   Prepare as directed under Tablets, with Morphine Hydrochloride Hydrate.

Identification   Weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 296 nm and 300 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 Morphine Hydrochloride Tablets add exactly 1 mL of the internal standard solution per 2 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$.HCl.3H$_2$O), disperse the tablet into a small particles using ultrasonic waves,

then treat with ultrasonic waves for 15 minutes with occasional stirring, and add water to make V mL so that each mL contains about 0.4 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$.HCl.3H$_2$O). Filter the solution, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$.HCl.3H$_2$O)
$$= M_S \times Q_T/Q_S \times V/50 \times 1.168$$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 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 15 minutes of Morphine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Morphine 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 morphine hydrochloride hydrate for assay (separately, determine the water <2.48> in the same manner as Morphine Hydrochloride Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 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 morphine in each solution.

Dissolution rate (%) with respect to the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$.HCl.3H$_2$O)
$$= M_S \times A_T/A_S \times 1/C \times 36 \times 1.168$$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$.HCl.3H$_2$O) 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 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of morphine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is not more than 2.0%.

Assay   Take not less than 20 Morphine Hydrochloride Tablets, weigh accurately, and powder. Weigh accurately a quantity of the powder, equivalent to about 20 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$.HCl.3H$_2$O), add exactly 10 mL of the internal standard solution, extract the mixture with ultrasonic waves for 10 minutes, and add water to make 50 mL. Filter this solution, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride hydrate for assay, dissolve in

exactly 10 mL of the internal standard solution, add water 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 morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride hydrate
($C_{17}H_{19}NO_3.HCl.3H_2O$)
= $M_S \times Q_T/Q_S \times 1.168$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 285 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, and mix.
Flow rate: Adjust so that the retention time of morphine 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, morphine 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 ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

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

Morphine and Atropine Injection

モルヒネ・アトロピン注射液

Morphine and Atropine Injection is an aqueous injection.

It contains not less than 0.91 w/v% and not more than 1.09 w/v% of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83].

Method of preparation

Morphine Hydrochloride Hydrate	10 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a significant quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Morphine and Atropine Injection is a clear, colorless liquid.
It is gradually colored by light.
pH: 2.5 – 5.0

Identification To 2 mL of Morphine and Atropine Injection add 2 mL of ammonia TS, and extract with 10 mL of diethyl ether. Filter the extract with a filter paper, evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Separately, dissolve 0.1 g of morphine hydrochloride hydrate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (1). Separately, dissolve 3 mg of atropine sulfate hydrate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained 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 for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the two spots obtained from the sample solution show the same color tone and the same Rf value with either spot of orange color obtained from the standard solution (1) or the standard solution (2) (morphine and atropine).

Extractable volume <6.05> It meets the requirement.

Assay (1) Morphine hydrochloride hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride hydrate for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as 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, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride hydrate
($C_{17}H_{19}NO_3.HCl.3H_2O$)
= $M_S \times Q_T/Q_S \times 1.168$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 285 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 with sodium hydroxide TS to 3.0. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
Flow rate: Adjust so that the retention time of morphine 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, morphine 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 ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Atropine Sulfate RS (separately determine the loss on drying <2.41> under the same conditions as Atropine Sulfate Hydrate), and dissolve in water 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 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 areas of atropine to that of the internal standard.

$$\text{Amount (mg) of atropine sulfate hydrate}$$
$$[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$$
$$= M_S \times Q_T/Q_S \times 1/25 \times 1.027$$

M_S: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 12,500).

Operating conditions—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

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

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, morphine, the internal standard and atropine are eluted in this order, and the resolution between morphine and the internal standard is 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 ratios of the peak area of atropine 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.

Morphine Sulfate Hydrate

モルヒネ硫酸塩水和物
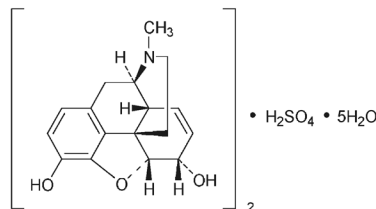
$(C_{17}H_{19}NO_3)_2.H_2SO_4.5H_2O$: 758.83
(5*R*,6*S*)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol hemisulfate hemipentahydrate
[6211-15-0]

Morphine Sulfate Hydrate contains not less than 98.0% and not more than 102.0% of morphine sulfate $[(C_{17}H_{19}NO_3)_2.H_2SO_4$: 668.75], calculated on the anhydrous basis.

Description   Morphine Sulfate Hydrate occurs as a white, crystals or crystalline powder.

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

It dissolves in dilute sodium hydroxide TS.

Identification   (1) Determine the absorption spectrum of a solution of Morphine Sulfate Hydrate (1 in 10,000) 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. Determine the absorption spectrum of a solution of Morphine Sulfate Hydrate in dilute sodium hydroxide TS (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.

(2) Determine the infrared absorption spectrum of Morphine Sulfate Hydrate 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) A solution of Morphine Sulfate Hydrate (1 in 25) responds to the Qualitative Tests <1.09> (1) and (3) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-107 - -112°$ (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

Purity   (1) Acidity—Dissolve 0.5 g of Morphine Sulfate Hydrate in 15 mL of water, add 2 drops of methyl red TS, and neutralize with 0.02 mol/L sodium hydroxide VS: the necessary volume of 0.02 mol/L sodium hydroxide VS is not more than 0.50 mL.

(2) Ammonium—Being specified separately when the drug is granted approval based on the Law.

(3) Chloride—Dissolve 0.10 g of Morphine Sulfate Hydrate in 10 mL of water, add 1 mL of dilute nitric acid, then add 1 mL of silver nitrate TS: no turbidity is produced.

(4) Meconic acid—Dissolve 0.20 g of Morphine Sulfate Hydrate in 5 mL of water, add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(5) Related substances—Dissolve 0.20 g of Morphine Sulfate Hydrate in 10 mL of diluted methanol (4 in 5), and use this solution as the sample solution. Pipet 1 mL of the

sample solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add diluted methanol (4 in 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 the 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 acetone, ethanol (99.5) and ammonia solution (28) (21:14:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot at Rf value of about 0.17 obtained with the sample solution is not more intense than the spot obtained with the standard solution (1), and the spot other than the principle spot, the spot at Rf value of about 0.17 and the spot at original point is not more intense than the spot with the standard solution (2).

Water <2.48>   11.0 – 13.0% (0.1 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 Morphine Sulfate Hydrate, dissolve in 3 mL of formic acid, add 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), 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.

$$\text{Each mL of 0.05 mol/L perchloric acid VS}$$
$$= 33.44 \text{ mg of } (C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$$

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

Mosapride Citrate Hydrate

モサプリドクエン酸塩水和物

and enantiomer
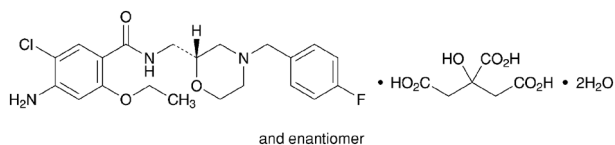
$C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7 \cdot 2H_2O$: 650.05
4-Amino-5-chloro-2-ethoxy-*N*-{[(2*RS*)-
4-(4-fluorobenzyl)morpholin-2-yl]methyl}benzamide
monocitrate dihydrate
[*636582-62-2*]

Mosapride Citrate Hydrate contains not less than 98.5% and not more than 101.0% of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$: 614.02), calculated on the anhydrous basis.

Description   Mosapride Citrate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide and in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Mosapride Citrate Hydrate in *N,N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification   **(1)**   Determine the absorption spectrum of a solution of Mosapride Citrate Hydrate 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.

(2)   Determine the infrared absorption spectrum of Mosapride Citrate 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 Mosapride Citrate Hydrate in *N,N*-dimethylformamide (1 in 10) responds to the Qualitative Tests <1.09> (1) for citrate.

Purity   **(1)**   Heavy metals <1.07>—Proceed with 1.0 g of Mosapride Citrate Hydrate in a platinum crucible 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 0.10 g of Mosapride Citrate Hydrate 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 50 mL. 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 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 by the automatic integration method: the area of the peak having the relative retention time of about 0.47 to mosapride from the sample solution is not larger than 3 times the peak area of mosapride from the standard solution, and the area of each peak other than mosapride and the peak mentioned above from the sample solution is not larger than the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than mosapride from the sample solution is not larger than 5 times the peak area of mosapride from the standard solution.

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 274 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: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 4.0 with dilute hydrochloric acid, and add water to make 1000 mL.
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 – 35	80 → 45	20 → 55

Flow rate: 1.0 mL per minute.
Time span of measurement: For 35 minutes after injection, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 4 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of mosapride obtained from 5 μL of this solution is equivalent to 15 to 25% 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 condi-

tions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40,000 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 mosapride is not more than 5.0%.

Water <*2.48*>   5.0 – 6.5% (0.5 g, volumetric titration, back titration).

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

Assay   Weigh accurately 0.5 g of Mosapride Citrate Hydrate, dissolve in 70 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.

$$\text{Each mL of } 0.1 \text{ mol/L perchloric acid VS}$$
$$= 61.40 \text{ mg of } C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$$

Containers and storage   Containers—Well-closed containers.

Mosapride Citrate Powder

モサプリドクエン酸塩散

Mosapride Citrate Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$: 614.02).

Method of preparation   Prepare as directed under Granules or Powders, with Mosapride Citrate Hydrate.

Identification   (1)   Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff's TS: an orange precipitate is formed.

(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 271 nm and 275 nm and between 306 nm and 310 nm.

Purity   Related substances—Powder Mosapride Citrate Powder. To a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), moisten with 1 mL of water, then add 9 mL of methanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol 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 two peaks, having the relative retention time of about 0.60 and about 0.85 to mosapride obtained from the sample solution, is not larger than the peak area of mosapride obtained from the standard solution, the area of other than mosapride and the peaks mentioned above is not larger than 2/5 times the peak area of mosapride from the standard solution, and the total area of the peak other than mosapride is not larger than 2

times the peak area of mosapride from the standard solution.
Operating conditions—

Detector, column, column temperature, mobile phases A and B, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.

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 – 40	85 – 45	15 – 55

Time span of measurement: For 40 minutes after injection, beginning after the solvent peak.
System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained with 10 μL of this solution is equivalent to 3.0 to 5.0% 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 mosapride 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 mosapride is not more than 3.0%.

Uniformity of dosage units <*6.02*>   Perform the test according to the following method: the powder in single-dose packages meets the requirement of the Content uniformity test.

To the total amount of the content of 1 package of Mosapride Citrate Powder add 5 mL of water, and shake. Then, add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL so that each mL contains about 20 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of mosapride citrate } (C_{21}H_{25}ClFN_3O_3.C_6H_8O_7)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/50$$

M_S: Amount (mg) of mosapride citrate 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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mosapride Citrate Powder is not less than 70%.

Start the test with an amount of Mosapride Citrate Powder, equivalent to about 2.5 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), 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 30 mg of mosapride citrate hydrate for assay (separately determine the water <*2.48*> in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2

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 50 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 mosapride in each solution.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
   = $M_S/M_T \times A_T/A_S \times 1/C \times 9$

M_S: Amount (mg) of mosapride citrate hydrate for assay taken, calculated on the anhydrous basis
M_T: Amount (g) of Mosapride Citrate Powder taken
C: Labeled amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) in 1 g

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 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 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust to pH 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile.

Flow rate: Adjust so that the retention time of mosapride is about 9 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 mosapride are not less than 4000 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 mosapride is not more than 2.0%.

Assay Powder Mosapride Citrate Powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), moisten with 2 mL of water, add 70 mL of methanol, shake for 20 minutes, then add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate hydrate for assay (separately determine the water <2.48> in the same manner as Mosapride Citrate Hydrate), and dissolve in methanol to make exactly 100 mL. To 2 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 the standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
   = $M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of mosapride citrate hydrate for assay taken, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Mosapride Citrate Tablets

モサプリドクエン酸塩錠

Mosapride Citrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$: 614.02).

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

Identification (1) To an amount of powdered Mosapride Citrate Tablets, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff's TS: an orange precipitate is formed.

(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 271 nm and 275 nm, and between 306 nm and 310 nm.

Purity Related substances—Powder not less than 20 tablets of Mosapride Citrate Tablets. Moisten a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), with 1 mL of water. Add 9 mL of methanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL. Pipet 2 mL of the sample solution, add methanol 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 peaks having the relative retention times of about 0.60 and about 0.85 to mosapride from the sample solution is not larger than the peak area of mosapride from the standard solution, and the area of each peak other than mosapride and these peaks mentioned above from the sample solution is not larger than 2/5 times the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than mosapride from the sample solution is not larger than 2 times the peak area of mosapride from the standard solution.
Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.

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 – 40	85 → 45	15 → 55

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 standard solution, and add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained from 10 μL of this solution is equivalent to 3.0 to 5.0% of that of mosapride

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 mosapride 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 mosapride is not more than 3.0%.

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 Mosapride Citrate Tablets add 5 mL of water, and shake well to disintegrate. Add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL so that each mL contains about 20 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
 = $M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of mosapride citrate 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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mosapride Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Mosapride 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 the dissolution medium to make exactly V' mL so that each mL contains about 2.8 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate hydrate for assay (separately, determine the water *<2.48>* in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 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 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 mosapride in each solution.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
  = $M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of mosapride citrate hydrate for assay taken, calculated on the anhydrous basis
C: Labeled amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 274 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 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile.
Flow rate: Adjust so that the retention time of mosapride is about 9 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 mosapride are not less than 4000 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 mosapride is not more than 2.0%.

Assay   Weigh accurately the mass of not less than 20 Mosapride Citrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$), and moisten with 2 mL of water. Add 70 mL of methanol, shake for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate hydrate for assay (separately, determine the water *<2.48>* in the manner as Mosapride Citrate Hydrate), and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol 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 Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 273 nm.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3.C_6H_8O_7$)
 = $M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of mosapride citrate hydrate for assay taken, calculated on the anhydrous basis

Containers and storage   Containers—Tight containers.

Freeze-dried Live Attenuated Mumps Vaccine

乾燥弱毒生おたふくかぜワクチン

Freeze-dried Live Attenuated Mumps Vaccine is a dried preparation containing live attenuated mumps virus.

It conforms to the requirements of Freeze-dried Live Attenuated Mumps Vaccine in the Minimum Requirements of Biologic Products.

Description   Freeze-dried Live Attenuated Mumps Vaccine becomes a clear, colorless, yellowish or reddish liquid on addition of solvent.

Mupirocin Calcium Hydrate

ムピロシンカルシウム水和物
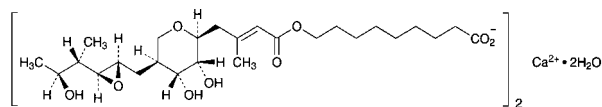
$C_{52}H_{86}CaO_{18}.2H_2O$: 1075.34
Monocalcium bis[9-((2*E*)-4-{(2*S*,3*R*,4*R*,5*S*)-5-
[(2*S*,3*S*,4*S*,5*S*)-2,3-epoxy-5-hydroxy-4-methylhexyl]-3,4-
dihydroxy-3,4,5,6-tetrahydro-2*H*-pyran-2-yl}-3-methylbut-
2-enoyloxy)nonanoate] dihydrate
[*115074-43-6*]

Mupirocin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Pseudomonas fluorescens*.

It contains not less than 895 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Mupirocin Calcium Hydrate is expressed as mass (potency) of mupirocin ($C_{26}H_{44}O_9$: 500.62).

Description Mupirocin Calcium Hydrate occurs as a white powder and has a bitter taste.

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

Identification (1) To 1 mL of a solution of Mupirocin Calcium Hydrate in methanol (1 in 200) add 4 mL of hydroxylamine perchlorate-ethanol TS and 1 mL of *N*,*N'*-dicyclohexylcarbodiimide-ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-ethanol TS to the solution, and shake: a dark purple color develops.

(2) Determine the absorption spectrum of a solution of Mupirocin Calcium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 219 nm and 224 nm.

(3) Determine the infrared absorption spectrum of Mupirocin Calcium Hydrate as directed in the paste method under Infrared Spectrophotometry *<2.25>*: it exhibits absorption at the wave numbers of about 1708 cm^{-1}, 1648 cm^{-1}, 1558 cm^{-1}, 1231 cm^{-1}, 1151cm^{-1} and 894 cm^{-1}.

(4) A solution of Mupirocin Calcium Hydrate (3 in 1000) responds to the Qualitative Tests *<1.09>* (3) for calcium salt.

Optical rotation *<2.49>* $[\alpha]_D^{20}$: $-16 - -20°$ (1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Related substances—Dissolve 50 mg of Mupirocin Calcium Hydrate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and a solution of tetrahydrofuran (3 in 4) (1:1) to make 10 mL, and use this solution as the sample solution (1). Pipet 2 mL of the sample solution (1), add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and a solution of tetra-hydrofuran (3 in 4) (1:1) to make 100 mL, and use this solution as the sample solution (2). Preserve these sample solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 μL of the sample solution (1) and the sample solution (2) as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the areas of each peak of the sample solution (1) and the sample solution (2) by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of principal related sub-stance (appeared at about 0.7 of the relative retention time to mupirocin) is not more than 4.0%, and the total amount of related substances (the total area of the peaks other than of the solvent and mupirocin) is not more than 6.0%.

Amount (%) of principal related substance

$$= \frac{A_i}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_m}}$$

Total amount (%) of related substances

$$= \frac{A}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_m}}$$

A: Total peak areas other than of the solvent and mupirocin from the sample solution (1)
A_i: Peak area of the relative retention time of about 0.7 to mupirocin from the sample solution (1)
A_m: A value of 50 times of peak area of mupirocin from the sample solution (2)
P: Potency per mg obtained from the assay

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 mupirocin, 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 sample solution (2), and add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained from 20 μL of this solution is equivalent to 4 to 6% of that obtained from 20 μL of the sample solution (2).

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution (2) under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 2.0%.

(2) Inorganic salt from manufacturing process—Being specified separately when the drug is granted approval based on the Law.

Water *<2.48>* Not less than 3.0% and not more than 4.5% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Mupirocin Calcium Hydrate and Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Preserve these solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 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 mupirocin in each solution.

Amount [μg (potency)] of mupirocin ($C_{26}H_{44}O_9$)
$= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Mupirocin Lithium RS 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 40°C.

Mobile phase: Dissolve 7.71 g of ammonium acetate in 750 mL of water, adjust the pH to 5.7 with acetic acid (100), and add water to make 1000 mL. To 300 mL of this solution add 100 mL of tetrahydrofuran.

Flow rate: Adjust so that the retention time of mupirocin is about 12.5 minutes.

System suitability—

System performance: Dissolve about 20 mg of Mupirocin Lithium RS and about 5 mg of ethyl parahydroxybenzoate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and a solution of tetrahydrofuran (3 in 4) (1:1) to make 200 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, mupirocin and ethyl parahydroxybenzoate 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 areas of mupirocin is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Mupirocin Calcium Ointment

ムピロシンカルシウム軟膏

Mupirocin Calcium Ointment is an oily ointment preparation.

Mupirocin Calcium Ointment contains not less than 95.0% and not more than 105.0% of the labeled potency of mupirocin ($C_{26}H_{44}O_9$: 500.62).

Method of preparation   Prepare as directed under Ointments, with Mupirocin Calcium Hydrate.

Identification   To an amount of Mupirocin Calcium Ointment, equivalent to 10 mg (potency) of Mupirocin Calcium Hydrate, add 5 mL of water, and warm on a water bath at 60°C for 10 minutes while occasional shaking. After cooling, filter, 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 a maximum between 220 nm and 224 nm.

Purity   Related substances—To an amount of Mupirocin Calcium Ointment, equivalent to 50 mg (potency) of Mupirocin Calcium Hydrate, add 5 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. Then, add 5 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0), shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and diluted tetrahydrofuran (3 in 4) (1:1) 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 area

of the peak other than mupirocin obtained from the sample solution and the peak area of mupirocin obtained from the standard solution by the automatic integration method. Calculate the amount of each related substance using the following equation: the amount of the related substance having the relative retention time of about 0.7 to mupirocin is not more than 4.0%, the amount of the related substance other than that is not more than 1.5%, and the total amount of the related substances is not more than 6.0%.

Amount (%) of each related substance
$$= A/(\Sigma A + A_m) \times 100$$

A: Peak area of each related substance obtained from the sample solution.

ΣA: Total area of the peaks other than mupirocin obtained from the sample solution.

A_m: Amount of 50 times the peak area of mupirocin 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 under Mupirocin Calcium Hydrate.

Time span of measurement: About 5 times as long as the retention time of mupirocin, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay under Mupirocin Calcium Hydrate.

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained with 20 μL of this solution is equivalent to 4 to 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 mupirocin is not more than 2.0%.

Assay   Weigh accurately an amount of Mupirocin Calcium Ointment, equivalent to about 2 mg (potency) of Mupirocin Calcium Hydrate, add exactly 10 mL of diluted tetrahydrofuran (3 in 4), and shake vigorously. To this solution add exactly 10 mL of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0), shake vigorously, filter through a glass wool filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Mupirocin Lithium RS, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) and diluted tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mupirocin Calcium Hydrate.
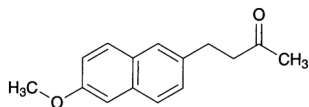
Amount [mg (potency)] of mupirocin ($C_{26}H_{44}O_9$)
$$= M_S \times A_T/A_S \times 1/10$$

M_S: Amount [mg (potency)] of Mupirocin Lithium RS taken

Containers and storage   Containers—Tight containers.

Nabumetone

ナブメトン

C$_{15}$H$_{16}$O$_2$: 228.29
4-(6-Methoxynaphthalen-2-yl)butan-2-one
[*42924-53-8*]

Nabumetone contains not less than 98.0% and not more than 101.0% of nabumetone (C$_{15}$H$_{16}$O$_2$), calculated on the anhydrous basis.

Description   Nabumetone occurs as white to yellowish white, crystals or a crystalline powder.

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

Identification   (1)   Determine the absorption spectrum of a solution of Nabumetone in methanol (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nabumetone 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 Nabumetone 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 Nabumetone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60>   79 – 84°C

Purity   (1)   Heavy metals <1.07>—Proceed with 1.0 g of Nabumetone 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 Nabumetone in 20 mL of acetonitrile, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetonitrile to make exactly 50 mL. Pipet 1 mL of this solution, add acetonitrile 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 of both solutions by the automatic integration method: the peak area of the related substance G obtained from the sample solution is not larger than 3/5 times the peak area of nabumetone obtained from the standard solution, and each peak area other than nabumetone and the related substance G is not larger than 1/5 times the peak area of nabumetone from the standard solution. Furthermore, the total area of the peaks other than nabumetone from the sample solution is not larger than 1.6 times the peak area of nabumetone from the standard solution. For each peak area of the related substances A, B, C, D, E, F and G, which are having the relative retention time of about 0.73, 0.85, 0.93, 1.2, 1.9, 2.6 and 2.7 to nabumetone, multiply their relative response factors, 0.12, 0.94, 0.25, 0.42, 1.02, 0.91 and 0.1, respectively.
Operating conditions—
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: A mixture of water and acetic acid (100) (999:1).

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

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 – 12	60	40
12 – 28	60 → 20	40 → 80

Flow rate: 1.3 mL per minute.

Time span of measurement: About 3 times as long as the retention time of nabumetone, 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 10 mL. Confirm that the peak area of nabumetone obtained from 10 μL of this solution is equivalent to 14 to 26% 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 nabumetone is not more than 5.0%.

Water <2.48>   Not more than 0.2% (1 g, volumetric titration, direct titration).

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

Assay   Weigh accurately about 20 mg each of Nabumetone and Nabumetone RS (separately determine the water <2.48> in the same manner as Nabumetone), dissolve them in acetonitrile to make exactly 20 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 area, A_T and A_S, of nabumetone in each solution.

Amount (mg) of nabumetone (C$_{15}$H$_{16}$O$_2$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Nabumetone 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 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

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

Mobile phase: To 600 mL of a mixture of water and acetic acid (100) (999:1) add 400 mL of a mixture of acetonitrile and tetrahydrofuran (7:3).

Flow rate: Adjust so that the retention time of nabumetone 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 number of theoretical plates and the symmetry factor of the peak of nabumetone 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 nabumetone is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Nabumetone Tablets

ナブメトン錠

Nabumetone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nabumetone ($C_{15}H_{16}O_2$: 228.29).

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

Identification   To a quantity of powdered Nabumetone Tablets, equivalent to 80 mg of Nabumetone, add 50 mL of methanol, shake for 10 minutes and centrifuge the solution. To 1 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 maxima between 259 nm and 263 nm, between 268 nm and 272 nm, between 316 nm and 320 nm, and between 330 nm and 334 nm.

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 a solution prepared by dissolving 3 g of polysorbate 80 in water to make 100 mL as the dissolution medium, the dissolution rate in 60 minutes of Nabumetone Tablets is not less than 70%.

Start the test with 1 tablet of Nabumetone 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 a solution, prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL, to make exactly V' mL so that each mL contains about 89 μg of nabumetone ($C_{15}H_{16}O_2$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Nabumetone RS (separately determine the water <2.48> in the same manner as Nabumetone), and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 331 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL as the blank.

Dissolution rate (%) with respect to the labeled amount of nabumetone ($C_{15}H_{16}O_2$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 360$$

M_S: Amount (mg) of Nabumetone RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of nabumetone ($C_{15}H_{16}O_2$) in 1 tablet

Assay   Weigh accurately the mass of not less than 20 tablets

of Nabumetone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.2 g of nabumetone ($C_{15}H_{16}O_2$), add 10 mL of water and shake, add 40 mL of methanol, shake for 30 minutes, and then add methanol to make exactly 100 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, 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 40 mg of Nabumetone RS (separately determine the water <2.48> in the same manner as Nabumetone), dissolve by adding 50 mL of methanol and exactly 20 mL of the internal standard solution, then add methanol 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 nabumetone to that of the internal standard.

Amount (mg) of nabumetone ($C_{15}H_{16}O_2$)
$$= M_S \times Q_T/Q_S \times 5$$

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

Internal standard solution—Dissolve 0.12 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.
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 25°C.
Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (550:450:1).
Flow rate: Adjust so that the retention time of nabumetone 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, nabumetone and the internal standard 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nabumetone to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Nadolol

ナドロール

and enantiomer
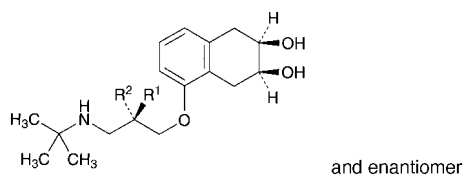
$C_{17}H_{27}NO_4$: 309.40
$R^1 = OH$, $R^2 = H$
(2*RS*,3*SR*)-5-{(2*SR*)-3-[(1,1-Dimethylethyl)amino]-
2-hydroxypropyloxy}-1,2,3,4-tetrahydronaphthalene-
2,3-diol

$R^1 = H$, $R^2 = OH$
(2*RS*,3*SR*)-5-{(2*RS*)-3-[(1,1-Dimethylethyl)amino]-
2-hydroxypropyloxy}-1,2,3,4-tetrahydronaphthalene-
2,3-diol
[*42200-33-9*]

Nadolol, when dried, contains not less than 98.0% of nadolol ($C_{17}H_{27}NO_4$).

Description Nadolol occurs as a white to yellow-brownish white crystalline powder.

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

A solution of Nadolol in methanol (1 in 100) shows no optical rotation.

Melting point: about 137°C.

Identification **(1)** Determine the absorption spectrum of a solution of Nadolol in methanol (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 wavelengths.

(2) Determine the infrared absorption spectrum of Nadolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1585 cm^{-1}, 1460 cm^{-1}, 1092 cm^{-1}, 935 cm^{-1} and 770 cm^{-1}.

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Nadolol 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 0.5 g of Nadolol in 10 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 100 μL each of the sample solution and a mixture of methanol and chloroform (1:1) as a control solution with 25 mm each of width at an interval of about 10 mm on the starting line of a plate 0.25 mm in thickness of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, chloroform and diluted ammonia TS (1 in 3) (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm), and confirm the positions of the principal spot and the spots other than the principal spot from the sample solution. Scratch and collect the silica gel of the positions of the plate corresponding to the principal spot and the spots other than the principal spot.

To the silica gel collected from the principal spot add exactly 30 mL of ethanol (95), and to the silica gel from the spots other than the principal spot add exactly 10 mL of ethanol (95). After shaking them for 60 minutes, centrifuge, and determine the absorbances of these supernatant liquids at 278 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. Separately, proceed in the same manner with each position of the silica gel from the control solution corresponding to the principal spot and the spots other than the principal spot of the sample solution, and perform a blank determination to make correction. Amount of the related substances calculated by the following equation is not more than 2.0%.

Amount (%) of related substances = $A_b/(A_b + 3A_a) \times 100$

A_a: Corrected absorbance of the principle spot
A_b: Corrected absorbance of the spots other than the principle spot

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 0.1% (1 g).

Isomer ratio Prepare a paste with 0.01 g of Nadolol as directed in the paste method under Infrared Spectrophotometry <2.25> so that its transmittance at an absorption band at a wave number of about 1585 cm^{-1} is 25 to 30%, and determine the infrared absorption spectrum between 1600 cm^{-1} and 1100 cm^{-1}. Determine the absorbances, A_{1265} and A_{1250}, from the transmittances, T_{1265} and T_{1250}, at wave numbers of about 1265 cm^{-1} (racemic substance A) and 1250 cm^{-1} (racemic substance B), respectively: the ratio A_{1265}/A_{1250} is between 0.72 and 1.08.

Assay Weigh accurately about 0.28 g of Nadolol, 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 purple through blue to green-blue (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
= 30.94 mg of $C_{17}H_{27}NO_4$

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

Nafamostat Mesilate

ナファモスタットメシル酸塩
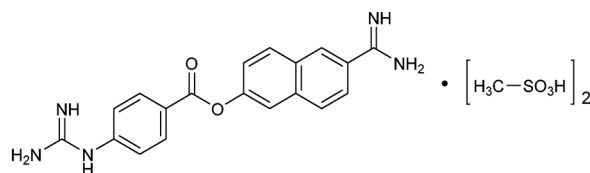
$C_{19}H_{17}N_5O_2.2CH_4O_3S$: 539.58
6-Amidinonaphthalen-2-yl 4-guanidinobenzoate
bis(methanesulfonate)
[*82956-11-4*]

Nafamostat Mesilate, when dried, contains not less than 99.0% and not more than 101.0% of nafamostat mesilate ($C_{19}H_{17}N_5O_2.2CH_4O_3S$).

Description Nafamostat Mesilate occurs as a white crystalline powder.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

Melting point: about 262°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Nafamostat Mesilate in 0.01 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.

(2) Determine the infrared absorption spectrum of Nafamostat 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.

(3) A 0.1-g portion of Nafamostat Mesilate responds to the Qualitative Tests <1.09> (1) for mesilate.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is between 4.7 and 5.7.

Purity (1) Clarity and color of solution—A solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Nafamostat 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).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Nafamostat Mesilate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mobile phase to make exactly 100 mL. Then 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 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 nafamostat obtained from the sample solution is not larger than 1/5 times the peak area of nafamostat obtained from the standard solution. Furthermore, the total area of the peaks other than nafamostat from the sample solution is not larger than the peak area of nafamostat 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 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 6.07 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (3 in 500). To 700 mL of this solution add 300 mL of acetonitrile.

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

Time span of measurement: About 4 times as long as the retention time of nafamostat, 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 50 mL. Pipet 15 mL of this solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of nafamostat obtained from 10 µL of this solution is equivalent to 1.1 to 1.9% of that obtained from 10 µL of the standard solution.

System performance: Dissolve 0.1 g of nafamostat mesilate in the mobile phase to make 100 mL. To 10 mL of this solution add the mobile phase to make 100 mL. To 5 mL of this solution add 5 mL of a solution of 6-amidino-2-naphthol methanesulfonate in the mobile phase (1 in 20,000). When the procedure is run with 10 µL of this solution under the above operating conditions, 6-amidino-2-naphthol and nafamostat 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 peak area of nafamostat 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 0.25 g of Nafamostat Mesilate, previously dried, dissolve in 4 mL of formic acid, add 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
= 26.98 mg of $C_{19}H_{17}N_5O_2 \cdot 2CH_4O_3S$

Containers and storage Containers—Tight containers.

Naftopidil

ナフトピジル
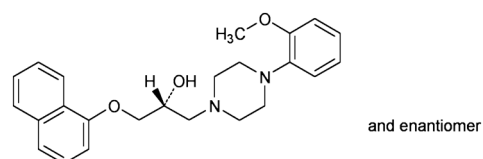
$C_{24}H_{28}N_2O_3$: 392.49
(2*RS*)-1-[4-(2-Methoxyphenyl)piperazin-1-yl]-3-(naphthalen-1-yloxy)propan-2-ol
[*57149-07-2*]

Naftopidil, when dried, contains not less than 99.0% and not more than 101.0% of naftopidil ($C_{24}H_{28}N_2O_3$).

Description Naftopidil occurs as a white crystalline powder.

It is very soluble in acetic anhydride, freely soluble in *N*,*N*-dimethylformamide and in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually colored to light brown by light.

A solution of Naftopidil in *N*,*N*-dimethylformamide (1 in 10) shows no optical rotation.

Identification (1) Dissolve 50 mg of Naftopidil in 5 mL of acetic acid (100), and add 0.1 mL of Dragendorff's TS: orange colored precipitates are produced.

(2) Determine the absorption spectrum of a solution of Naftopidil in methanol (1 in 100,000) as directed under Ul-

traviolet-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 Naftopidil, 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>   126 – 129°C

Purity   (1) Heavy metals <1.07>—Proceed with 2.0 g of Naftopidil 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 0.10 g of Naftopidil in 60 mL of methanol, add diluted 0.1 mol/L potassium dihydrogen phosphate TS (pH 2.0) (1 in 2) to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and water (3:2) to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and water (3:2) 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 automatic integration method: each peak area other than naftopidil obtained from the sample solution is not larger than 3/4 times the peak area of naftopidil obtained from the standard solution, and the total area of the peaks other than naftopidil from the sample solution is not larger than 2.5 times the peak area of naftopidil from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 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 (5 μm in particle diameter).

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

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 450 mL of this solution add 550 mL of methanol.

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

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

System suitability—

Test for required detectability: Pipet 2.5 mL of the standard solution, add a mixture of methanol and water (3:2) to make exactly 10 mL. Confirm that the peak area of naftopidil obtained with 10 μL of this solution is equivalent to 17.5 to 32.5% 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 naftopidil are not less than 2500 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 naftopidil is not more than 3.0%.

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 0.2 g of Naftopidil, 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
= 39.25 mg of $C_{24}H_{28}N_2O_3$

Containers and storage   Containers—Well-closed containers.
Storage—Light-resistant.

Naftopidil Orally Disintegrating Tablets

ナフトピジル口腔内崩壊錠

Naftopidil Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of naftopidil ($C_{24}H_{28}N_2O_3$: 392.49).

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

Identification   Powder Naftopidil Orally Disintegrating Tablets. To a portion of the powder, equivalent to 25 mg of Naftopidil add 100 mL of methanol, shake thoroughly, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 6 mL of the filtrate add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 281 nm and 285 nm, and between 318 nm and 322 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 Naftopidil Orally Disintegrating Tablets add V/10 mL of water, disintegrate and disperse the tablet with the aid of ultrasonic waves. To this solution add V/2 mL of methanol, shake thoroughly, then add methanol to make exactly V mL so that each mL contains about 0.25 mg of naftopidil ($C_{24}H_{28}N_2O_3$), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 6 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in methanol to make exactly 100 mL. Pipet 3 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, at 283 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$)
= $M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of naftopidil for assay taken

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

Dissolution <6.10>   When the test is performed at 50 revolu-

tion 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 30 minutes of Naftopidil Orally Disintegrating Tablets is not less than 75%.

Start the test with 1 tablet of Naftopidil 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 28 μg of naftopidil ($C_{24}H_{28}N_2O_3$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, then add the dissolution medium to make exactly 100 mL. Pipet 5 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 283 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 naftopidil ($C_{24}H_{28}N_2O_3$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$$

M_S: Amount (mg) of naftopidil for assay taken
C: Labeled amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Naftopidil Orally Disintegrating Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of naftopidil ($C_{24}H_{28}N_2O_3$), add 30 mL of methanol, shake thoroughly, add diluted 0.1 mol/L potassium dihydrogen phosphate TS (pH 2.0) (1 in 2) to make exactly 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 30 mL of methanol, add diluted 0.1 mol/L potassium dihydrogen phosphate TS (pH 2.0) (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) 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 naftopidil to that of the internal standard.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of naftopidil for assay taken

Internal standard solution—A solution of butyl para-hydroxybenzoate in a mixture of methanol and water (3:2) (3 in 2000).
Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Naftopidil.
System suitability—
System performance: When the procedure is run with 10

μL of the standard solution under the above operating conditions, naftopidil 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 ratio of the peak area of naftopidil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Naftopidil Tablets

ナフトピジル錠

Naftopidil Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of naftopidil ($C_{24}H_{28}N_2O_3$: 392.49).

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

Identification Powder Naftopidil Tablets. To a portion of the powder, equivalent to 25 mg of Naftopidil, add 100 mL of methanol, shake thoroughly, and centrifuge, if necessary. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To 6 mL of the filtrate add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 281 nm and 285 nm, and between 318 nm and 322 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 Naftopidil Tablets add V/10 mL of water, disintegrate and disperse the tablet with the aid of ultrasonic waves. To this dispersed solution add V/2 mL of methanol, shake thoroughly, add methanol to make exactly V mL so that each mL contains about 0.25 mg of naftopidil ($C_{24}H_{28}N_2O_3$). Centrifuge this solution, if necessary, 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, pipet 6 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in methanol to make exactly 100 mL. Pipet 3 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, at 283 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$)
$$= M_S \times A_T/A_S \times V/200$$

M_S: Amount (mg) of naftopidil 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 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 15 minutes of 25-mg and 50-mg tablet and in 30 minutes of 75-mg tablet is not less than 75%.

Start the test with 1 tablet of Naftopidil 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 28 μg of naftopidil ($C_{24}H_{28}N_2O_3$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 50 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 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 283 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 naftopidil ($C_{24}H_{28}N_2O_3$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of naftopidil for assay taken
C: Labeled amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Naftopidil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of naftopidil ($C_{24}H_{28}N_2O_3$), add 30 mL of methanol, shake thoroughly, and add diluted 0.1 mol/L potassium dihydrogen phosphate TS (pH 2.0) (1 in 2) to make exactly 50 mL. Centrifuge this solution, if necessary, 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, pipet 10 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 30 mL of methanol, add diluted 0.1 mol/L potassium dihydrogen phosphate TS (pH 2.0) (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) 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 naftopidil to that of the internal standard.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of naftopidil for assay taken

Internal standard solution—A solution of butyl para-hydroxybenzoate in a mixture of methanol and water (3:2) (3 in 2000).
Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Naftopidil.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, naftopidil 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 ratio of
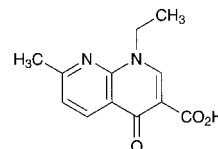
the peak area of naftopidil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Nalidixic Acid

ナリジクス酸

$C_{12}H_{12}N_2O_3$: 232.24
1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid
[389-08-2]

Nalidixic Acid, when dried, contains not less than 99.0% and not more than 101.0% of nalidixic acid ($C_{12}H_{12}N_2O_3$).

Description Nalidixic Acid occurs as white to light yellow, crystals or crystalline powder.
It is sparingly soluble in *N,N*-dimethylformamide, very slightly soluble in ethanol (99.5), and practically insoluble in water.
It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Nalidixic Acid in 0.01 mol/L sodium hydroxide 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.

(2) Determine the infrared absorption spectrum of Nalidixic 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.

Melting point <2.60> 225 – 231°C

Purity (1) Chloride <1.03>—To 2.0 g of Nalidixic Acid add 50 mL of water, warm at 70°C for 5 minutes, cool quickly, 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 with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.012%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Nalidixic 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).

(3) Related substances—Dissolve 20 mg of Nalidixic Acid in 20 mL of 0.01 mol/L sodium hydroxide TS. To 5 mL of this solution, add water to make 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 1000 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 nalidixic acid with the sample solution is not larger than the peak area of nalidixic acid with the standard solution, and the total area of the peaks other than nalidixic acid with the sample solution is not larger than 2.5 times the peak area of nalidixic acid with 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: Dissolve 6.24 g of sodium dihydrogen phosphate dihydrate in 950 mL of water, adjust the pH to 2.8 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of methanol.

Flow rate: Adjust so that the retention time of nalidixic acid is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of nalidixic acid, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of nalidixic acid 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: Dissolve 25 mg of methyl parahydroxybenzoate in 100 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add water to make 10 mL. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and nalidixic acid 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nalidixic acid is not more than 2.0%.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.3 g of Nalidixic Acid, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethyl ammonium hydroxide VS (potentiometric titration). Separately, to 50 mL of *N,N*-dimethylformamide add 13 mL of a mixture of water and methanol (89:11), perform a blank determination with the solution, and make any necessary correction.

Each mL of 0.1 mol/L tetramethyl ammonium hydroxide VS
  = 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and storage Containers—Tight containers.

Naloxone Hydrochloride

ナロキソン塩酸塩
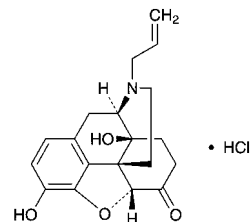
$C_{19}H_{21}NO_4.HCl$: 363.84
(5*R*,14*S*)-17-Allyl-4,5-epoxy-3,14-dihydroxymorphinan-6-one monohydrochloride
[*357-08-4*]

Naloxone Hydrochloride contains not less than 98.5% of naloxone hydrochloride ($C_{19}H_{21}NO_4.HCl$), calculated on the dried basis.

Description Naloxone Hydrochloride occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in acetic anhydride.

It is hygroscopic.

It is gradually colored by light.

Identification (1) Determine the absorption spectrum of a solution of Naloxone Hydrochloride (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 Naloxone 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 Naloxone Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{25}$: −170 – −181° (0.25 g calculated on the dried basis, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Naloxone Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 4.5 and 5.5.

Purity Related substances—Conduct this procedure as rapidly as possible without exposure to light, using light-resistant containers. Dissolve 0.08 g of Naloxone 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 with a mixture of ammonia-saturated 1-butanol TS and methanol (20:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly iron (III) chloride-potassium hexacyanoferrate (III) TS on the plate: the number of the spot other than the principal spot from the sample solution is not more than 1 and it is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 2.0% [0.1 g, 105°C, 5 hours. Use a desiccator (phosphorus (V) oxide) for cooling].

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

Assay Weigh accurately about 0.3 g of Naloxone Hydrochloride, dissolve in 80 mL of acetic acid (100) by warming. After cooling, add 80 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.
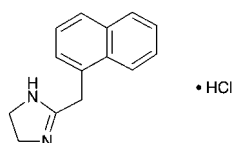
$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 36.38 \text{ mg of } C_{19}H_{21}NO_4 \cdot HCl$$

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

Naphazoline Hydrochloride

ナファゾリン塩酸塩

$C_{14}H_{14}N_2 \cdot HCl$: 246.74
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole
monohydrochloride
[*550-99-2*]

Naphazoline Hydrochloride, when dried, contains not less than 98.5% of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$).

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

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

Melting point: 255 – 260°C (with decomposition).

Identification **(1)** To 10 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 30 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Evaporate the combined diethyl ether extracts to dryness with the aid of a current of air. Dry the residue at 80°C for 1 hour: the residue melts <2.60> between 117°C and 120°C.

(3) Dissolve 0.02 g of the residue obtained in (2) in 2 to 3 drops of dilute hydrochloric acid and 5 mL of water, and add 2 mL of Reinecke salt TS: a red-purple, crystalline precipitate is formed.

(4) A solution of Naphazoline Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.10 g of Naphazoline Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

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

(2) Heavy metals <1.07>—Proceed with 1.0 g of Naphazoline 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% (1 g, 105°C, 2 hours).

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

Assay Weigh accurately about 0.4 g of Naphazoline 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.
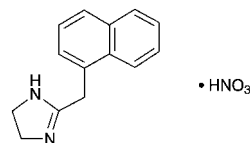
$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 24.67 \text{ mg of } C_{14}H_{14}N_2 \cdot HCl$$

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

Naphazoline Nitrate

ナファゾリン硝酸塩

$C_{14}H_{14}N_2 \cdot HNO_3$: 273.29
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole
mononitrate
[*5144-52-5*]

Naphazoline Nitrate, when dried, contains not less than 98.5% of naphazoline nitrate ($C_{14}H_{14}N_2 \cdot HNO_3$).

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

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

Identification **(1)** To 10 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 20 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness with the aid of a current of air, and dry the residue at 80°C for 1 hour: the residue so obtained melts <2.60> between 117°C and 120°C.

(3) A solution of Naphazoline Nitrate (1 in 20) responds to the Qualitative Tests <1.09> for nitrate.

pH <2.54> Dissolve 0.1 g of Naphazoline Nitrate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

Melting point <2.60> 167 – 170°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Naphazoline Nitrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Naphazoline Nitrate 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% (1 g, 105°C,

2 hours).

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

Assay   Weigh accurately about 0.4 g of Naphazoline Nitrate, 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 (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
= 27.33 mg of $C_{14}H_{14}N_2.HNO_3$

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

Naphazoline and Chlorpheniramine Solution

ナファゾリン・クロルフェニラミン液

Naphazoline and Chlorpheniramine Solution contains not less than 0.045 w/v% and not more than 0.055 w/v% of naphazoline nitrate ($C_{14}H_{14}N_2.HNO_3$: 273.29), and not less than 0.09 w/v% and not more than 0.11 w/v% of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86).

Method of preparation

Naphazoline Nitrate	0.5 g
Chlorpheniramine Maleate	1 g
Chlorobutanol	2 g
Glycerin	50 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Dissolve, and mix the above ingredients.

Description   Naphazoline and Chlorpheniramine Solution is a clear, colorless liquid.

Identification   (1)   To 20 mL of Naphazoline and Chlorpheniramine Solution add 2 mL of a solution of potassium hydroxide (7 in 10) and 5 mL of pyridine, and heat at 100°C for 5 minutes: a red color is produced (chlorobutanol).

(2)   Place 10 mL of Naphazoline and Chlorpheniramine Solution in a glass-stoppered test tube, add 10 mL of ethanol (95), 2 mL of sodium hydroxide TS and 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color is produced (glycerin).

(3)   To 20 mL of Naphazoline and Chlorpheniramine Solution add 5 mL of sodium hydroxide TS, extract with 10 mL of diethyl ether, and separate the diethyl ether layer. Take 5 mL of this solution, distil off the solvent, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.01 g each of naphazoline nitrate and Chlorpheniramine Maleate RS in 10 mL and 5 mL of methanol, respectively, 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 on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wave-length: 254 nm): two spots from the sample solution exhibit the same Rf values as the spots from standard solutions (1) and (2). Spray evenly Dragendorff's TS on the plate: the spots from standard solutions (1) and (2) and the corresponding spot from the sample solutions reveal an orange color.

Assay   Pipet 4 mL of Naphazoline and Chlorpheniramine Solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of naphazoline nitrate for assay, dried at 105°C for 2 hours, and about 0.1 g of Chlorpheniramine Maleate RS, dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 4 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 solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak height of naphazoline and chlorpheniramine to that of the internal standard of the standard solution.

Amount (mg) of naphazoline nitrate ($C_{14}H_{14}N_2.HNO_3$)
= $M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/25$

Amount (mg) of chlorpheniramine maleate
($C_{16}H_{19}ClN_2.C_4H_4O_4$)
= $M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/25$

M_{Sa}: Amount (mg) of naphazoline nitrate for assay taken
M_{Sb}: Amount (mg) of Chlorpheniramine Maleate RS taken

Internal standard solution—A solution of ethenzamide in methanol (1 in 1000).
Operating conditions—
   Detector: An ultraviolet absorption photometer (wave-length: 254 nm).
   Column: A stainless steel column, about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 μm in particle diameter).
   Column temperature: Room temperature.
   Mobile phase: A mixture of acetonitrile and a solution of sodium laurylsulfate (1 in 500) in diluted phosphoric acid (1 in 1000) (1:1).
   Flow rate: Adjust so that the retention time of chlorpheniramine is about 10 minutes.
   Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of the internal standard, naphazoline and chlorpheniramine in this order.

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

Naproxen

ナプロキセン
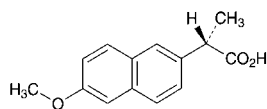
C$_{14}$H$_{14}$O$_3$: 230.26
(2*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid
[*22204-53-1*]

Naproxen, when dried, contains not less than 98.5% of naproxen (C$_{14}$H$_{14}$O$_3$).

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

It is freely soluble in acetone, soluble in methanol, in ethanol (99.5) and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 0.01 g of Naproxen in 5 mL of methanol, add 5 mL of water, then add 2 mL of potassium iodide TS and 5 mL of a solution of potassium iodate (1 in 100), and shake: a yellow to yellow-brown color develops. To this solution add 5 mL of chloroform, and shake: a light red-purple color develops in the chloroform layer.

(2) To 1 mL of a solution of Naproxen in ethanol (99.5) (1 in 300) add 4 mL of hydroxylamine perchlorate-ethanol TS and 1 mL of *N,N'*-dicyclohexylcarbodiimide-ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-ethanol TS, and shake: a red-purple color develops.

(3) Determine the absorption spectrum of a solution of Naproxen in ethanol (99.5) (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.

(4) Determine the infrared absorption spectrum of Naproxen, 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> [α]$_D^{25}$: +63.0 – +68.5° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 154 – 158°C

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Naproxen in 20 mL of acetone: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 400 nm is not more than 0.070.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Naproxen 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 Naproxen according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Naproxen in 10 mL of a mixture of ethanol (99.5) and chloroform (1:1), and use this solution as the sample so-

lution. Pipet 2 mL of the sample solution, and add a mixture of ethanol (99.5) and chloroform (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of ethanol (99.5) and chloroform (1:1) 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 hexane, dichloromethane, tetrahydrofuran and acetic acid (100) (50:30:17:3) 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 and the spot of the starting 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% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.5g of Naproxen, previously dried, add 100 mL of diluted methanol (4 in 5), dissolve by gentle warming if necessary, 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
= 23.03 mg of C$_{14}$H$_{14}$O$_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nartograstim (Genetical Recombination)

ナルトグラスチム（遺伝子組換え）

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MAPTYRASSL PQSFLLKSLE QVRKIQGDGA ALQEKLCATY KLCHPEELVL

LGHSLGIPWA PLSSCPSQAL QLAGCLSQLH SGLFLYQGLL QALEGISPEL

GPTLDTLQLD VADFATTIWQ QMEELGMAPA LQPTQGAMPA FASAFQRRAG

GVLVASHLQS FLEVSYRVLR HLAQP
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C$_{850}$H$_{1344}$N$_{226}$O$_{245}$S$_8$: 18905.65
[*134088-74-7*]

Nartograstim (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human granulocyte colony-stimulating factor (G-CSF) analog. It is *N*-methionylated, and threonine, leucine, glycine, proline and cysteine residues at the positions, 1, 3, 4, 5 and 17 of G-CSF are substituted by alanine, threonine, tyrosine, arginine and serine, respectively. It is a glycoprotein consisting of 175 amino acid residues. It has a stimulating effect on neutrophil production.

It contains not less than 0.9 mg and not more than 2.1 mg of protein per mL, and not less than 4.0 × 10^8 units per mg of protein.

Description Nartograstim (Genetical Recombination) occurs as a clear and colorless, liquid.

Identification (1) To a suitable amount of Nartograstim (Genetical Recombination) add tris-sodium chloride buffer solution (pH 8.0) so that each mL contains 1 μg of protein,

and use this solution as the sample solution. Put 0.1 mL of the sample solution in the well of a microplate for antigen-antibody reaction test, allow to stand at 5°C for not less than 10 hours, then remove the liquid, and wash the well. Then to the well add 0.25 mL of blocking TS for nartograstim test, and allow to stand at room temperature for 1 hour. Remove the blocking TS, add 0.1 mL of rabbit anti-nartograstim antibody TS to the well, and stir gently at room temperature for 3 hours. Remove the rabbit anti-nartograstim antibody TS, and wash the well. Then, add 0.1 mL of peroxidase labeled anti-rabbit antibody TS, stir gently at room temperature for 2 hours, remove the TS, and wash the well. Then, add 0.1 mL of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt TS, allow to stand at room temperature for 10 minutes, add 0.1 mL of a solution of oxalic acid dihydrate (1 in 50), and name this well as the sample well. Separately, proceed with 0.1 mL of tris-sodium chloride buffer solution (pH 8.0) in the same manner as for the sample solution, and name the well so obtained as the control well. When compare the sample well and the control well, the sample well reveals a green color, while the control well reveals no color.

Washing procedure of well: To the well add 0.25 mL of washing fluid for nartograstim test, allow to stand for 3 minutes, and remove the washing fluid. Repeat this procedure 2 times more.

(2) To a suitable amount of Nartograstim (Genetical Recombination) add water so that each mL contains 1 mg of protein. Replace the solvent of 2 mL of this solution with tris-calcium chloride buffer solution (pH 6.5). To 0.5 mL of the solution so obtained add 0.5 mL of tris-calcium chloride buffer solution (pH 6.5) and 5 μL of thermolysin solution (1 in 1000), allow to stand at 37°C for 21 hours, and use this solution as the sample solution. Separately, proceed with 2 mL of Nartograstim RS in the same manner as for the sample solution, and use the solution so obtained 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 these chromatograms: the similar peaks appear at the same retention times.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 35°C.

Mobile phase A: A mixture of water and trifluoroacetic acid (1000:1).

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (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 – 5	100	0
5 – 90	100 → 40	0 → 60

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above conditions, the number of the peak which shows not less than 1.6 of the resolution between the adjacent peaks is not less than 15.

Molecular mass To a suitable amount of Nartograstim (Genetical Recombination) add reduction buffer solution for nartograstim sample so that each mL contains about 0.5 mg of protein, and use this solution as the sample solution. Separately, to 50 μL of molecular mass marker for nartograstim test add reduction buffer solution for nartograstim sample to make 1.0 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution, both previously warmed at 40°C for 15 minutes, by SDS polyacrylamide gel electrophoresis, using buffer solution for SDS polyacrylamide gel electrophoresis and polyacrylamide gel for nartograstim. After electrophoresis, immerse the gel in a solution of coomassie brilliant blue R-250 in a mixture of water, ethanol (95) and acetic acid (100) (5:4:1) (1 in 1000), and stir gently at room temperature for not less than 12 hours. Then, destain the gel with a mixture of water, ethanol (95) and acetic acid (100) (13:5:2), and dry the gel under reduced pressure. Prepare a calibration curve from the migration distance of the molecular mass markers of the standard solution by plotting the migration distance on the horizontal axis and logarithm of the molecular mass on the vertical axis. Calculate the molecular mass of the sample solution from the calibration curve: the molecular mass of the main band is between 17,000 and 19,000.

Compositions ratio of related substance Being specified separately when the drug is granted approval based on the Law.

pH *<2.54>*   7.0 – 7.5

Purity **(1)** Related substances—To a suitable amount of Nartograstim (Genetical Recombination) add the buffer solution for nartograstim sample so that each mL contains about 0.5 mg of protein, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the buffer solution for nartograstim sample 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 by SDS polyacrylamide gel electrophoresis, using buffer solution for SDS polyacrylamide gel electrophoresis and polyacrylamide gel for nartograstim. After electrophoresis, immerse the gel in a solution of coomassie brilliant blue R-250 in a mixture of water, ethanol (95) and acetic acid (100) (5:4:1) (1 in 1000), and stir gently at room temperature for not less than 12 hours. Then, destain the gel with a mixture of water, ethanol (95) and acetic acid (100) (13:5:2), and dry the gel under reduced pressure. Determine the areas of the colored bands obtained from the sample solution and standard solution by a densitometer at the measure wavelength 560 nm and the control wavelength 400 nm: the total area of the band other than the principal band obtained from the sample solution is not larger than the band area obtained from the standard solution.

(2) Host cell protein—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.

Bacterial endotoxins *<4.01>*   Less than 0.62 EU/μg.

Assay **(1)** Protein content—To exactly V_1 mL of Nartograstim (Genetical Recombination) add exactly V_2 mL of water so that each mL contains about 0.5 mg of protein, and centrifuge. Determine the absorbance, A, of the supernatant liquid at the absorption maximum at about 280 nm as di-

rected under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of protein in 1 mL of Nartograstim
(Genetical Recombination)
$$= A/8.71 \times (V_1 + V_2)/V_1 \times 10$$

8.71: Specific absorbance

(2) Specific activity—To a suitable exact amount of Nartograstim (Genetical Recombination) add potency measuring medium for nartograstim test so that the potency is equivalent to 50% to 150% of the relative potency of the standard solution according to the expected potency, and use this solution as the sample solution. Separately, to a suitable exact amount of Nartograstim RS add an exact amount of the potency measuring medium for nartograstim test so that each mL contains exactly 1.2×10^4 units of nartograstim, and use this solution as the standard solution. Culture NFS-60 cells with subculture medium for nartograstim test, centrifuge the medium, remove the supernatant liquid by suction, and wash the cells with the potency measuring medium for nartograstim test. Repeat the washing procedure twice more, prepare two cell suspensions, containing 8×10^5 cells per mL and 4×10^5 cells per mL in the potency measuring medium for nartograstim test, and use these solutions as the cell suspension (1) and (2), respectively. In 8 wells of the 12th column of a 8×12 well-microplate put $50\,\mu$L each of the cell suspension (1), and in all wells of the 1st to 11th columns put $50\,\mu$L each of the cell suspension (2). Where, the wells of the 1st and 8th lines are not used for the test. To the wells of the 2nd to 4th lines of the 12th column add 50 μL each of the standard solution, and to the wells of 5th to 7th lines of the 12th column add $50\,\mu$L each of the sample solution. From the wells of the 12th column take $50\,\mu$L each of the content liquid and transfer to the corresponding wells of the 1st column. Then, from the wells of the 1st column take $50\,\mu$L each of the content liquid and transfer to the corresponding wells of the 2nd column. Proceed in the same way sequentially to the 10th column to prepare two-fold serial dilution wells. The wells of the 11th column are not performed any process. Incubate the plate under the atmosphere of 5 vol% carbon dioxide at 37°C for about 40 hours. After incubation, add to the all wells 10 μL each of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide TS, and allow to stand under the atmosphere of 5 vol% carbon dioxide at 37°C for 4 – 6 hours. Add 0.125 mL of dimethylsulfoxide, shake for 5 to 10 minutes, then determine the absorbances of all wells at 550 nm and 660 nm, A_1 and A_2, using a spectrophotometer for microplate, and calculate the difference, $(A_1 - A_2)$. Divide by 6 the total of the differences $(A_1 - A_2)$ of six wells of the 11th and the 1st column, which were added the standard solution, and use the value so obtained as the 50% absorbance, A_M. Determine the dilution index numbers (column number) of the two serial wells of the sample solution and standard solution, they are corresponding to just the before and after of the 50% absorbance (A_M), n_{T1}, n_{T2} and n_{S1}, n_{S2}, respectively, where $n_{T1} < n_{T2}$ and $n_{S1} < n_{S2}$. Differences of the absorbance of the serial wells are named as A_{T1}, A_{T2} and A_{S1}, A_{S2}, respectively. Calculate the relative potencies of each sample solution by the following equation using the mean value of the three standard solutions, and average them. Perform the same procedure by reversing the place of the sample solution and the standard solution. Then, calculate the mean relative potency by averaging both values.

$$\text{Relative potency of the sample solution} = \frac{2^a}{\Sigma 2^b \times \dfrac{1}{3}}$$

a: $n_{T1} + (A_{T1} - A_M)/(A_{T1} - A_{T2})$
b: $n_{S1} + (A_{S1} - A_M)/(A_{S1} - A_{S2})$

Obtain the potency per mL by the following equation, and calculate the potency per mg of protein using the protein content obtained in (1).

Amount (unit) of nartograstim per mL of Nartograstim (Genetical Recombination)
$$= S \times \text{mean relative potency of the sample solution} \times d$$

S: Concentration (unit/mL) of the standard solution
d: Dilution factor for the sample solution

System suitability—
The absorbance difference of the individual wells of the standard solution of the 3rd column should be not less than A_M, and that of the individual wells of the 8th column should be not more than A_M. If they do not meet the requirements, prepare the standard solution of the range of 1.0×10^3 to 1.6×10^4 units, and perform the test.

Containers and storage Containers—Tight containers.
Storage—Not exceeding −20°C.

Nartograstim for Injection (Genetical Recombination)

注射用ナルトグラスチム（遺伝子組換え）

Nartograstim for Injection (Genetical Recombination) 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 nartograstim (genetical recombination) ($C_{850}H_{1344}N_{226}O_{245}S_8$: 18905.65).

Method of preparation Prepare as directed under Injections, with Nartograstim (Genetical Recombination).

Description Nartograstim for Injection (Genetical Recombination) occurs as white, masses or powder.

Identification Dissolve the content of 1 package of Nartograstim for Injection (Genetical Recombination) in 1 mL of tris-sodium chloride buffer solution (pH 8.0). To a suitable amount of this solution add tris-sodium chloride buffer solution (pH 8.0) so that each mL contains 1 μg of Nartograstim (Genetical Recombination), and use this solution as the sample solution. Then, proceed with the sample solution as directed in the Identification (1) under Nartograstim (Genetical Recombination).

pH <2.54> Being specified separately when the drug is granted approval based on the Law.

Purity **(1) Clarity and color of solution**—A solution of Nartograstim for Injection (Genetical Recombination) in water, containing 100 μg of Nartograstim (Genetical Recombination) in each mL, is clear and colorless.
(2) Lactose conjugate—Being specified separately when the drug is granted approval based on the Law.

Water <2.48> Not more than 3.0% (50 mg, coulometric titration).

Bacterial endotoxins <4.01> Less than 0.62 EU/μg.

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, using 3 mL of water for injection per 1 Nartograstim for Injection (Genetical Recombination) to dissolve the content: 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, using the sample solution prepared by dissolving the sample in water in a concentration to be used for the injection: it meets the requirement.

Specific activity　Nartograstim for Injection (Genetical Recombination), when perform the assay and the following test, contains not less than 4.0×10^8 units of nartograstim (genetical recombination) per mg nartograstim (genetical recombination).

Wash out each content of 10 Nartograstim for Injection (Genetical Recombination) with a suitable amount of potency measuring medium for nartograstim test, wash the empty containers with the same medium, combine all washings, and add the same medium to make exactly 50 mL. To an exact amount of this solution add the same medium so that the concentration of nartograstim (genetical recombination) is equivalent to 50% to 150% of that of the standard solution, and use this solution as the sample solution. Separately, weigh accurately a suitable amount of Nartograstim RS, dissolve in the potency measuring medium for nartograstim test so that each mL contains exactly 1.2×10^4 units of nartograstim according to the labeled unit, and use this solution as the standard solution. Then, determine the nartograstim potency (unit) in 1 Nartograstim for Injection (Genetical Recombination) by proceeding as directed in the Assay (2) under Nartograstim (Genetical Recombination), and calculate the ratio against the amount of nartograstim obtained in the Assay.

Nartograstim (genetical recombination) potency (unit) in 1 Nartograstim for Injection (Genetical Recombination)

$$= S \times \text{mean relative potency of the sample solution} \times d \times 5$$

S: Concentration (unit/mL) of the standard solution
d: Dilution factor for the sample solution
5: Amount (mL) of the medium used to dissolve per 1 sample

$$\text{Relative activity of sample solution} = \frac{\Sigma 2^a}{\Sigma 2^b \times \dfrac{1}{3}}$$

a: $n_{T1} + (A_{T1} - A_M)/(A_{T1} - A_{T2})$
b: $n_{S1} + (A_{S1} - A_M)/(A_{S1} - A_{S2})$

System suitability—
Proceed as directed in the system suitability in the Assay (2) under Nartograstim (Genetical Recombination).

Assay　Weigh accurately the mass of each content of not less than 10 Nartograstim for Injection (Genetical Recombination). Weigh accurately an amount of the content, equivalent to about 0.25 mg of Nartograstim (Genetical Recombination), dissolve in exactly 5 mL of the mobile phase, and use this solution as the sample solution. Separately, dissolve a suitable amount of Nartograstim RS in the mobile phase so that each mL contains about 50 μg of nartograstim, 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 nartograstim in each solution.

Amount (μg) of nartograstim (genetical recombination) in 1 Nartograstim for Injection (Genetical Recombination)

$$= M_S \times A_T/A_S \times M/M_T \times 5$$

M_S: Amount (μg) of nartograstim in 1 mL of the standard solution
M: Mean mass (mg) of each content
M_T: Amount (mg) of Nartograstim for Injection (Genetical Recombination) taken

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with porous silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate and 1.0 g of sodium lauryl sulfate in 700 mL of water, adjust to pH 6.5 with sodium hydroxide TS, and add water to make 1000 mL.
Flow rate: Adjust so that the retention time of nartograstim is about 16 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 nartograstim are not less than 3000 and not more than 2.0, respectively.
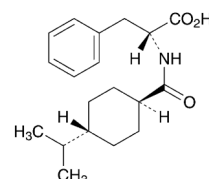System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nartograstim is not more than 1.5%.

Containers and storage　Containers—Hermetic containers.
Storage—Light-resistant, and at a temperature not exceeding 10°C.

Nateglinide

ナテグリニド

C₁₉H₂₇NO₃: 317.42
N-[*trans*-4-(1-Methylethyl)cyclohexanecarbonyl]-D-phenylalanine
[*105816-04-4*]

Nateglinide, when dried, contains not less than 98.0% and not more than 102.0% of nateglinide ($C_{19}H_{27}NO_3$).

Description　Nateglinide occurs as a white crystalline powder.
It is freely soluble in methanol and in ethanol (99.5), sparingly soluble in acetonitrile, and practically insoluble in water.
It dissolves in dilute sodium hydroxide TS.
It shows crystal polymorphism.

Identification　(1)　Determine the absorption spectrum of a solution of Nateglinide in methanol (1 in 1000) as directed

under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nateglinide 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 Nateglinide 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 Nateglinide 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 reference standard according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-36.5 - -40.0°$ (after drying, 0.2 g, dilute sodium hydroxide TS, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nateglinide 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 0.25 g of Nateglinide in 20 mL of acetonitrile. To 4 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add the mobile phase to make exactly 50 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 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 nateglinide from the sample solution is not larger than the peak area of nateglinide 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 4 times as long as the retention time of nateglinide, 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 nateglinide are not less than 6000 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 nateglinide is not more than 2.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, 105°C, 2 hours).

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

Assay Weigh accurately about 0.1 g of Nateglinide, previously dried, and dissolve in acetonitrile to make exactly 20 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 Nateglinide RS, previously dried, and dissolve in acetonitrile to make exactly 20 mL. Pipet 10 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 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 nateglinide to that of the internal standard.

$$\text{Amount (mg) of nateglinide (C}_{19}\text{H}_{27}\text{NO}_3)$$
$$= M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of Nateglinide RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 500).
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 40°C.
  Mobile phase: Adjust 0.05 mol/L sodium dihydrogen phosphate TS to pH 2.5 with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.
  Flow rate: Adjust so that the retention time of nateglinide 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 nateglinide 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nateglinide Tablets

ナテグリニド錠

Nateglinide Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of nateglinide (C$_{19}$H$_{27}$NO$_3$: 317.42).

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

Identification To an amount of powdered Nateglinide Tablets, equivalent to 20 mg of Nateglinide, add 20 mL of methanol, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 246 nm and 250 nm, between 251 nm and 255 nm, between 257 nm and 261 nm and between 262 nm and 266 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 Nateglinide Tablets add 10 mL of 0.05 mol/L sodium dihydrogen phosphate TS adjusted to pH 2.5 with phosphoric acid, shake to disintegrate the tablet, and disperse to fine particles with the aid of ultrasonic waves.

Add exactly $3V/50$ mL of the internal standard solution, add $3V/5$ mL of acetonitrile, shake for 10 minutes, and add acetonitrile to make V mL so that each mL contains about 0.6 mg of nateglinide ($C_{19}H_{27}NO_3$). Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm, and discard the first 5 mL of the filtrate. To 8 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Nateglinide RS, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 10 mL. Pipet 6 mL of this solution, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. To 8 mL of this solution add 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>, and calculate the ratios, Q_T and Q_S, of the peak area of nateglinide to that of the internal standard.

$$\text{Amount (mg) of nateglinide } (C_{19}H_{27}NO_3)$$
$$= M_S \times Q_T/Q_S \times 3V/250$$

M_S: Amount (mg) of Nateglinide RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 250).
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 nateglinide 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard 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 45 minutes of a 30-mg tablet and that in 30 minutes of a 90-mg tablet of Nateglinide Tablets is not less than 75%, respectively.

Start the test with 1 tablet of Nateglinide 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 33 μg of nateglinide ($C_{19}H_{27}NO_3$), and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Nateglinide RS, previously dried at 105°C for 2 hours, and dissolve in acetonitrile 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, and determine the peak areas, A_T and A_S, of nateglinide in each solution.

Dissolution rate (%) with respect to the labeled amount of nateglinide ($C_{19}H_{27}NO_3$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$$

M_S: Amount (mg) of Nateglinide RS taken
C: Labeled amount (mg) of nateglinide ($C_{19}H_{27}NO_3$) 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 nateglinide are not less than 8000 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 nateglinide is not more than 2.0%.

Assay   To 20 Nateglinide Tablets add $V/5$ mL of 0.05 mol/L sodium dihydrogen phosphate TS adjusted to pH 2.5 with phosphoric acid, shake to disintegrate the tablets, and disperse to fine particles with the aid of ultrasonic waves. Then, add $V/2$ mL of acetonitrile and exactly $V/10$ mL of the internal standard solution, shake for 10 minutes, and add acetonitrile to make V mL so that each mL contains about 6 mg of nateglinide ($C_{19}H_{27}NO_3$). 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 4 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 60 mg of Nateglinide RS, previously dried at 105°C for 2 hours, add exactly 1 mL of the internal standard solution, and add acetonitrile to make 10 mL. To 4 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, and calculate the ratios, Q_T and Q_S, of the peak area of nateglinide to that of the internal standard.

$$\text{Amount (mg) of nateglinide } (C_{19}H_{27}NO_3) \text{ in 1 tablet}$$
$$= M_S \times Q_T/Q_S \times V/200$$

M_S: Amount (mg) of Nateglinide RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (3 in 125).
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 40°C.
Mobile phase: Adjust to pH 2.5 of 0.05 mol/L sodium dihydrogen phosphate TS with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile.
Flow rate: Adjust so that the retention time of nateglinide 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 nateglinide are eluted in this order with the resolution between these peaks being not less than 19.
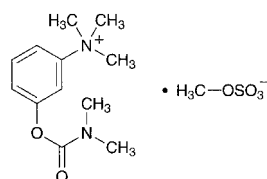System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the ratio of the peak area of nateglinide to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Neostigmine Methylsulfate

ネオスチグミンメチル硫酸塩

C$_{13}$H$_{22}$N$_2$O$_6$S: 334.39
3-(Dimethylcarbamoyloxy)-*N*,*N*,*N*-
trimethylanilinium methyl sulfate
[*51-60-5*]

Neostigmine Methylsulfate, when dried, contains not less than 98.0% and not more than 102.0% of neostigmine methylsulfate (C$_{13}$H$_{22}$N$_2$O$_6$S).

Description   Neostigmine Methylsulfate occurs as a white crystalline powder.

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

Identification   **(1)**   Determine the absorption spectrum of a solution of Neostigmine Methylsulfate (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Neostigmine Methylsulfate 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 Neostigmine Methylsulfate 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 Neostigmine Methylsulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*>   Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.0 and 5.0.

Melting point <*2.60*>   145 – 149°C

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

(2)   Sulfate—Dissolve 0.20 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

(3)   Dimethylaminophenol—Dissolve 0.10 g of Neostigmine Methylsulfate in 5 mL of water, add 1 mL of sodium hydroxide TS, and while cooling with ice, add 1 mL of diazobenzenesulfonic acid TS: no color develops.

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 25 mg each of Neostigmine Methylsulfate and Neostigmine Methylsulfate RS, previously dried, dissolve each 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 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 neostigmine in each solution.

Amount (mg) of neostigmine methylsulfate (C$_{13}$H$_{22}$N$_2$O$_6$S)
    = $M_S \times A_T/A_S$

M_S: Amount (mg) of Neostigmine Methylsulfate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 259 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 3.12 g of sodium dihydrogenphosphate dihydrate in 1000 mL of water, adjust to pH 3.0 with phosphoric acid, and add 0.871 g of sodium 1-pentanesulfonate to dissolve. To 890 mL of this solution add 110 mL of acetonitrile.

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

System suitability—

System performance: Dissolve 25 mg of Neostigmine Methylsulfate and 4 mg of dimethylaminophenol in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, dimethylaminophenol and neostigmine 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 peak areas of neostigmine methylsulfate is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Neostigmine Methylsulfate Injection

ネオスチグミンメチル硫酸塩注射液

Neostigmine Methylsulfate Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of neostigmine methylsulfate (C$_{13}$H$_{22}$N$_2$O$_6$S: 334.39).

Method of preparation   Prepare as directed under Injections, with Neostigmine Methylsulfate.

Description   Neostigmine Methylsulfate Injection is a clear, colorless liquid.

It is slowly affected by light.

pH: 5.0 – 6.5

Identification   Take a volume of Neostigmine Methylsulfate Injection, equivalent to 5 mg of neostigmine methylsulfate, add water to make 10 mL if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 257 nm and 261 nm.

Bacterial endotoxins <4.01> Less than 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 Use Neostigmine Methylsulfate Injection as the sample solution. Separately, weigh accurately about 25 mg of Neostigmine Methylsulfate RS, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Neostigmine Methylsulfate.
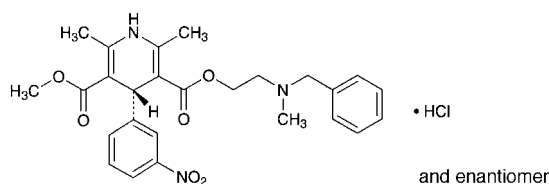
Amount (mg) of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$)
  $= M_S \times A_T/A_S$

M_S: Amount (mg) of Neostigmine Methylsulfate RS taken

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

Nicardipine Hydrochloride

ニカルジピン塩酸塩

• HCl

and enantiomer

$C_{26}H_{29}N_3O_6 \cdot HCl$: 515.99
2-[Benzyl(methyl)amino]ethyl methyl (4*RS*)-
2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-
3,5-dicarboxylate monohydrochloride
[*54527-84-3*]

Nicardipine hydrochloride, when dried, contains not less than 98.5% of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$).

Description Nicardipine Hydrochloride occurs as a pale greenish yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water, in acetonitrile and in acetic anhydride.

A solution of Nicardipine Hydrochloride in methanol (1 in 20) shows no optical rotation.

It is gradually affected by light.

Identification (1) Determine the absorption spectrum of a solution of Nicardipine Hydrochloride 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 Nicardipine 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) Dissolve 0.02 g of Nicardipine Hydrochloride in 10

mL of water and 3 mL of nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 167 – 171°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Nicardipine 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).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Nicardipine 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 50 mL, then take exactly 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 of both solutions by the automatic integration method: the area of each peak other than nicardipine from the sample solution is not larger than the peak area of nicardipine from the standard solution, and the total area of each peak other than nicardipine is not larger than 2 times the peak area of nicardipine 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 a solution of perchloric acid (43 in 50,000) and acetonitrile (3:2).
Flow rate: Adjust so that the retention time of nicardipine is about 6 minutes.
Time span of measurement: About 4 times as long as the retention time of nicardipine, 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 20 mL. Confirm that the peak area of nicardipine 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 2 mg each of Nicardipine Hydrochloride and nifedipine in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, nicardipine and nifedipine 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 areas of nicardipine is not more than 3%.

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 Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.9 g of Nicardipine Hydrochloride, 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 a blank determination,

and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.60 mg of $C_{26}H_{29}N_3O_6 \cdot HCl$

Containers and storage   Containers—Well-closed containers.

Storage—Light-resistant.

Nicardipine Hydrochloride Injection

ニカルジピン塩酸塩注射液

Nicardipine Hydrochloride Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$: 515.99).

Method of preparation   Prepare as directed under Injections, with Nicardipine Hydrochloride.

Description   Nicardipine Hydrochloride Injection occurs as a clear, pale yellow liquid.

It is gradually changed by light.

Identification   To a volume of Nicardipine Hydrochloride Injection, equivalent to 1 mg of Nicardipine Hydrochloride, add ethanol (99.5) 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, and between 351 nm and 355 nm.

pH <2.54>   3.0 – 4.5

Purity   Related substances—Conduct the procedure without exposure to light, using light-resistant vessels. To a volume of Nicardipine Hydrochloride Injection, equivalent to 5 mg of Nicardipine Hydrochloride, add the mobile phase to make 10 mL, and use this solution as the sample solution. To exactly 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 the peak areas of these solutions by the automatic integration method: the areas of the peaks other than nicardipine from the sample solution are not larger than the peak area of nicardipine from the standard solution, and the total of the areas of the peaks other than nicardipine is not larger than 2 times of the peak area of nicardipine 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 nicardipine, 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 mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that obtained from 10 μL of the standard solution.

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 areas of nicardipine is not more than 1.0%.

Bacterial endotoxins <4.01>   Less than 8.33 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 the procedure without exposure to light, using light-resistant vessels. To an exact volume of Nicardipine Hydrochloride Injection, equivalent to about 2 mg of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nicardipine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and 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 nicardipine to that of the internal standard.

Amount (mg) of nicardipine hydrochloride
($C_{26}H_{29}N_3O_6 \cdot HCl$)
= $M_S \times Q_T/Q_S \times 1/25$

M_S: Amount (mg) of nicardipine hydrochloride for assay taken

Internal standard solution—A solution of di-*n*-butyl phthalate in methanol (1 in 625).
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 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL. To 320 mL of this solution add 680 mL of methanol.
Flow rate: Adjust so that the retention time of nicardipine 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, nicardipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.
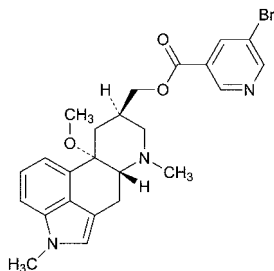System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

Containers and storage   Containers—Hermetic containers. Colored containers may be used.
Storage—Light-resistant.

Nicergoline

ニセルゴリン

C$_{24}$H$_{26}$BrN$_3$O$_3$: 484.39
[(8*R*,10*S*)-10-Methoxy-1,6-dimethylergolin-8-yl]methyl
5-bromopyridine-3-carboxylate
[*27848-84-6*]

Nicergoline, when dried, contains not less than 98.5% and not more than 101.0% of nicergoline (C$_{24}$H$_{26}$BrN$_3$O$_3$).

Description　Nicergoline occurs as white to light yellow, crystals or crystalline powder.

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

It is gradually colored to light brown by light.

Melting point: about 136°C (with decomposition).

Identification　**(1)**　Determine the absorption spectrum of a solution of Nicergoline 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 Nicergoline 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›*　[α]$_D^{20}$: +5.2 – +6.2° (after drying, 0.5 g, ethanol (95), 10 mL, 100 mm).

Purity　**(1)**　Heavy metals *‹1.07›*—Proceed with 2.0 g of Nicergoline 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 25 mg of Nicergoline in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile 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 by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 to nicergoline from the sample solution, is not larger than 4 times the peak area of nicergoline from the standard solution, and the area of the peak other than nicergoline and the peak mentioned above from the sample solution is not larger than 2.5 times the peak area of nicergoline from the standard solution. The peak which area is larger than the peak area of nicergoline from the standard solution is not more than two peaks, and the total area of the peaks other than nicergoline from the

sample solution is not larger than 7.5 times the peak area of nicergoline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 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: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust so that the retention time of nicergoline is about 25 minutes.

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

System suitability—

Test for required detectability: To 1 mL of the sample solution add acetonitrile to make exactly 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 acetonitrile to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% 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 sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline 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 nicergoline is not more than 4.0%.

Loss on drying *‹2.41›*　Not more than 0.5% (2 g, in vacuum, 60°C, 2 hours).

Residue on ignition *‹2.44›*　Not more than 0.1% (1 g).

Assay　Weigh accurately about 0.4 g of Nicergoline, previously dried, add 10 mL of acetic anhydride, and warm to dissolve. After cooling, add 40 mL of nitrobenzene, and titrate *‹2.50›* with 0.1 mol/L perchloric acid VS until the color of the solution changes to blue-green from red through a blue-purple (indicator: 10 drops of neutral red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.22 mg of C$_{24}$H$_{26}$BrN$_3$O$_3$

Containers and storage　Containers—Well-closed containers.

Storage—Light-resistant.

Nicergoline Powder

ニセルゴリン散

Nicergoline Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$: 484.39).

Method of preparation　Prepare as directed under Granules or Powders, with Nicergoline.

Identification　Vigorously shake for 10 minutes a quantity of Nicergoline Powder, equivalent to 10 mg of Nicergoline, with 20 mL of diluted ethanol (4 in 5), and centrifuge for 10 minutes. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

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 substances other than nicergoline by the area percentage method: the total amount of them 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 2 times as long as the retention time of nicergoline, 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 standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) 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, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that obtained with 20 μL of the solution for system suitability test.
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 nicergoline is not more than 1.5%.

Uniformity of dosage units <6.02>　The Nicergoline Powder 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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Nicergoline Powder is not less than 80%.

Start the test with an accurately weighed amount of Nicergoline Powder, equivalent to about 5 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a laminated polyester fiber. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 225 nm, A_{T1} and A_{S1}, and at 250 nm, A_{T2} and A_{S2}, 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 nicergoline ($C_{24}H_{26}BrN_3O_3$)
$$= M_S/M_T \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times 1/C \times 9$$

M_S: Amount (mg) of nicergoline for assay taken
M_T: Amount (g) of Nicergoline Powder taken
C: Labeled amount (mg) of nicergoline ($C_{24}H_{26}BrN_3O_3$) in 1 g

Assay　Weigh accurately a quantity of Nicergoline Powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), 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 nicergoline in each solution.

Amount (mg) of nicergoline ($C_{24}H_{26}BrN_3O_3$)
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of nicergoline for assay taken

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 288 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: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.
Flow rate: Adjust so that the retention time of nicergoline is about 25 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 nicergoline 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 nicergoline is not more than 1.0%.

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

Nicergoline Tablets

ニセルゴリン錠

Nicergoline Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$: 484.39).

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

Identification   Take a quantity of powdered Nicergoline Tablets, equivalent to 10 mg of Nicergoline, add 20 mL of ethanol (99.5), shake vigorously for 10 minutes, and filter through a 0.45-μm pore-size membrane filter. To 2 mL of the filtrate add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

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 substances other than nicergoline by the area percentage method: the total amount of them 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 2 times as long as the retention time of nicergoline, 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 standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) 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, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that obtained with 20 μL of the solution for system suitability test.
   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 nicergoline is not more than 1.5%.

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 Nicergoline Tablets add exactly 25 mL of diluted ethanol (4 in 5), disperse to fine particles with the aid of ultrasonic wave, and shake for 5 minutes. Centrifuge this solution for 10 minutes, pipet 4 mL of the supernatant liquid, add diluted ethanol (4 in 5) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in exactly 25 mL of diluted ethanol (4 in 5). Pipet 4 mL of this solution, add diluted ethanol (4 in 5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, A_{T1} and A_{S1}, and at 340 nm, A_{T2} and

A_{S2}, of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_3O_3)$$
$$= M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times 1/2$$

M_S: Amount (mg) of nicergoline for assay taken

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

Assay   Weigh accurately the mass of not less than 20 Nicergoline Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), 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 nicergoline in each solution.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_3O_3) = M_S \times A_T/A_S$$

M_S: Amount (mg) of nicergoline for assay taken

Operating conditions—
   Detector: An ultraviolet absorption photometer (wavelength: 288 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: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.
   Flow rate: Adjust so that the retention time of nicergoline is about 25 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 nicergoline 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 nicergoline is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Niceritrol

ニセリトロール
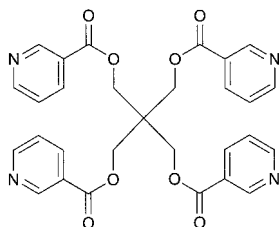
C$_{29}$H$_{24}$N$_4$O$_8$: 556.52
Pentaerythritol tetranicotinate
[*5868-05-3*]

Niceritrol, when dried, contains not less than 99.0% of niceritrol (C$_{29}$H$_{24}$N$_4$O$_8$).

Description Niceritrol occurs as a white to pale yellowish white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, soluble in *N,N*-dimethylformamide, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Niceritrol 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 Niceritrol, 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>* 162 – 165°C

Purity (1) Chloride *<1.03>*—To 2.0 g of Niceritrol add 50 mL of water, and warm at 70°C for 20 minutes, while shaking occasionally. After cooling, filter, and 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 1.0 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 Niceritrol 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 Niceritrol 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).

(4) Pyridine—Dissolve 0.5 g of Niceritrol in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add *N,N*-dimethylformamide to make exactly 100 mL, then pipet 0.5 mL of this solution, add *N,N*-dimethylformamide to make exactly 10 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 Gas Chromatography *<2.02>* according to the following conditions. Determine each peak area of pyridine in both solutions: the

peak area of pyridine from the sample solution is not larger than the peak area of pyridine from the standard solution.
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A column 3 mm in inside diameter and 3 m in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 10% on acid-treated and silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter).
Column temperature: A constant temperature of about 160°C.
Carrier gas: Nitrogen.
Flow rate: Adjust so that the retention time of pyridine is about 2 minutes.
System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of pyridine is not less than 1500.
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 areas of pyridine is not more than 3.0%.

(5) Free acids—Transfer about 1 g of Niceritrol, weighed accurately, to a separator, dissolve in 20 mL of chloroform, and extract with 20 mL and then 10 mL of water while shaking well. Combine the whole extracts, and titrate *<2.50>* with 0.01 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, make any necessary correction, and calculate the amount of free acid by the following equation: it is not more than 0.1%.

Each mL of 0.01 mol/L sodium hydroxide VS
= 1.231 mg of C$_6$H$_5$NO$_2$

(6) Related substances—Dissolve 0.10 g of Niceritrol in 10 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 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 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) (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% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 1 g of Niceritrol, previously dried, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, boil gently for 20 minutes under a reflux condenser with a carbon dioxide absorber (soda lime). After cooling, titrate *<2.50>* immediately the excess sodium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 69.57 mg of C$_{29}$H$_{24}$N$_4$O$_8$

Containers and storage Containers—Well-closed containers.

Nicomol

ニコモール
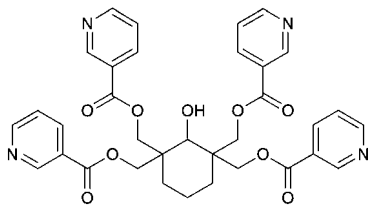
C$_{34}$H$_{32}$N$_4$O$_9$: 640.64
(2-Hydroxycyclohexane-1,1,3,3-tetrayl)tetramethyl
tetranicotinate
[*27959-26-8*]

Nicomol, when dried, contains not less than 98.0% of nicomol (C$_{34}$H$_{32}$N$_4$O$_9$).

Description Nicomol occurs as a white crystalline powder. It is odorless and tasteless.

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

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Identification   (1) Mix 0.01 g of Nicomol with 0.02 g of 1-chloro-2,4-dinitrobenzene, add 2 mL of dilute ethanol, heat in a water bath for 5 minutes, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Dissolve 0.1 g of Nicomol in 5 mL of dilute hydrochloric acid, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Nicomol 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.

(4) Determine the infrared absorption spectrum of Nicomol, 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>   181 – 185°C

Purity   (1) Clarity and color of solution—Dissolve 1.0 g of Nicomol in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Nicomol add 50 mL of freshly boiled and cooled water, shake for 5 minutes, filter, and to 25 mL of the filtrate add 0.60 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(3) Chloride <1.03>—Dissolve 0.6 g of Nicomol in 15 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 as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 15 mL of dilute nitric acid and water to make 50 mL (not more than 0.024%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Nicomol 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 Nicomol according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of Nicomol 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 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 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, ethanol (95), acetonitrile and ethyl acetate (5:3:1:1) 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>   Not more than 1.0% (1 g, 105°C, 4 hours).

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

Assay Weigh accurately about 1.5 g of Nicomol, previously dried, add exactly 40 mL of 0.5 mol/L sodium hydroxide VS, and boil gently under a reflux condenser connected to a carbon dioxide absorption tube (soda lime) for 10 minutes. After cooling, titrate <2.50> immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 80.08 mg of C$_{34}$H$_{32}$N$_4$O$_9$

Containers and storage Containers—Tight containers.

Nicomol Tablets

ニコモール錠

Nicomol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicomol (C$_{34}$H$_{32}$N$_4$O$_9$: 640.64).

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

Identification To a portion of powdered Nicomol Tablets, equivalent to 0.5 g of Nicomol, add 20 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) and (2) under Nicomol.

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 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Nicomol Tablets is not less than 75%.

Start the test with 1 tablet of Nicomol 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 contains about 18 μg of nicomol (C$_{34}$H$_{32}$N$_4$O$_9$), and use

this solution as the sample solution. Separately, weigh accurately about 0.1 g of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in the dissolution medium to make exactly 100 mL, then pipet 2 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 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of nicomol ($C_{34}H_{32}N_4O_9$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of nicomol for assay taken
C: Labeled amount (mg) of nicomol ($C_{34}H_{32}N_4O_9$) in 1 tablet

Assay Weigh accurately not less than 20 Nicomol Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of nicomol ($C_{34}H_{32}N_4O_9$), add 100 mL of 1 mol/L hydrochloric acid TS, shake well, add water to make exactly 500 mL, and filter. Discard the first 50 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 50 mL of 1 mol/L hydrochloric acid TS and water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in 50 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add 20 mL of 1 mol/L hydrochloric acid TS and 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 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

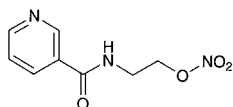Amount (mg) of nicomol ($C_{34}H_{32}N_4O_9$)
$$= M_S \times A_T/A_S \times 25/2$$

M_S: Amount (mg) of nicomol for assay taken

Containers and storage Containers—Tight containers.

Nicorandil

ニコランジル

$C_8H_9N_3O_4$: 211.17
N-[2-(Nitrooxy)ethyl]pyridine-3-carboxamide
[*65141-46-0*]

Nicorandil contains not less than 98.5% and not more than 101.0% of nicorandil ($C_8H_9N_3O_4$), calculated on the anhydrous basis.

Description Nicorandil occurs as white crystals.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), soluble in acetic anhydride, and sparingly soluble in water.

Melting point: about 92°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Nicorandil (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 Nicorandil 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) Sulfate <1.14>—Dissolve 2.0 g of Nicorandil in 20 mL of dilute ethanol, 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 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.010%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Nicorandil 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 20 mg of Nicorandil in 10 mL of the mobile phase, 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, and determine each peak area by the automatic integration method: the peak area of *N*-(2-hydroxyethyl)isonicotinamide nitric ester, having the relative retention time of about 0.86 to nicorandil, is not more than 0.5% of the peak area of nicorandil, the area of all other peaks is less than 0.1%, and the sum area of the peaks other than nicorandil and *N*-(2-hydroxyethyl)isonicotinamide nitric ester is not more than 0.25% of the total peak area.

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 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, triethylamine and trifluoroacetic acid (982:10:5:3).

Flow rate: Adjust so that the retention time of nicorandil is about 18 minutes.

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

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 500 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 20 mL. Confirm that the peak area of nicorandil obtained with 10 μL of this solution is equivalent to 2 to 8% of that obtained with 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of *N*-(2-hydroxyethyl)isonicotinamide nitric ester in the mobile phase to make 100 mL. To 1 mL of this solution add 10 mL of the sample solution. When the procedure is run with this solution under the above operating conditions, *N*-(2-hydroxyethyl)isonicotinamide nitric ester and nicorandil are eluted in this order with the resolution between these peaks being not less than 3.0.

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 nicorandil is not more than 1.5%.

Water ⟨*2.48*⟩   Not more than 0.1% (2 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 Nicorandil, 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.
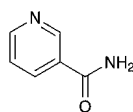
Each mL of 0.1 mol/L perchloric acid VS
= 21.12 mg of $C_8H_9N_3O_4$

Containers and storage   Containers—Tight containers.
Storage—At a temperature between 2°C and 8°C.

Nicotinamide

ニコチン酸アミド

$C_6H_6N_2O$: 122.12
Pyridine-3-carboxamide
[*98-92-0*]

Nicotinamide, when dried, contains not less than 98.5% and not more than 102.0% of nicotinamide ($C_6H_6N_2O$).

Description   Nicotinamide occurs as white, crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

Identification   **(1)**   Mix 5 mg of Nicotinamide with 0.01 g of 1-chloro-2,4-dinitrobenzene, heat gently for 5 to 6 seconds, and fuse the mixture. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a red color is produced.

(2)   To 0.02 g of Nicotinamide add 5 mL of sodium hydroxide TS, and boil carefully: the gas evolved turns moistened red litmus paper blue.

(3)   Dissolve 0.02 g of Nicotinamide in water to make 1000 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 Nicotinamide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH ⟨*2.54*⟩   Dissolve 1.0 g of Nicotinamide in 20 mL of water: the pH of this solution is between 6.0 and 7.5.

Melting point ⟨*2.60*⟩   128 – 131°C

Purity   **(1)**   Clarity and color of solution—Dissolve 1.0 g of Nicotinamide in 20 mL of water: the solution is clear and colorless.

(2)   Chloride ⟨*1.03*⟩—Take 0.5 g of Nicotinamide, and perform the test. 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*⟩—Take 1.0 g of Nicotinamide, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4)   Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Nicotin-

amide according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(5)   Readily carbonizable substances ⟨*1.15*⟩—Take 0.20 g of Nicotinamide, and perform the test. The solution has no more color than Matching Fluid A.

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 0.1% (1 g).

Assay   Weigh accurately about 25 mg each of Nicotinamide and Nicotinamide RS, both previously dried, dissolve separately in 3 mL of water, and add the mobile phase to make exactly 100 mL. Pipet 8 mL each of these solutions, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, 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 nicotinamide to that of the internal standard.

Amount (g) of nicotinamide ($C_6H_6N_2O$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of dried Nicotinamide RS taken

Internal standard solution—A solution of nicotinic acid (1 in 25,000).
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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
   Column temperature: A constant temperature of about 25°C.
   Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of methanol.
   Flow rate: Adjust so that the retention time of nicotinamide 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, the internal standard and nicotinamide 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 ratio of the peak area of nicotinamide to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Nicotinic Acid

ニコチン酸
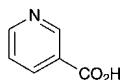
C$_6$H$_5$NO$_2$: 123.11
Pyridine-3-carboxylic acid
[*59-67-6*]

Nicotinic Acid, when dried, contains not less than 99.5% of nicotinic acid (C$_6$H$_5$NO$_2$).

Description Nicotinic Acid occurs as white, crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is sparingly soluble in water, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

Identification (1) Triturate 5 mg of Nicotinic Acid with 0.01 g of 1-chloro-2,4-dinitrobenzene, and fuse the mixture by gentle heating for 5 to 6 seconds. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color is produced.

(2) Dissolve 0.02 g of Nicotinic Acid in water to make 1000 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 Nicotinic Acid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH *<2.54>* Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the pH of this solution is between 3.0 and 4.0.

Melting point *<2.60>* 234 – 238°C

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the solution is clear and colorless.

(2) Chloride *<1.03>*—Perform the test with 0.5 g of Nicotinic Acid. 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 1.0 g of Nicotinic Acid in 3 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 and 3 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.019%).

(4) Nitro compounds—Dissolve 1.0 g of Nicotinic Acid in 8 mL of sodium hydroxide TS, and add water to make 20 mL: the solution has no more color than Matching Fluid A.

(5) Heavy metals *<1.07>*—Proceed with 1.0 g of Nicotinic 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).

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

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

Assay Weigh accurately about 0.3 g of Nicotinic Acid, previously dried, dissolve in 50 mL of water, and titrate *<2.50>* with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS).

<div style="text-align:center">

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.31 mg of C$_6$H$_5$NO$_2$

</div>

Containers and storage Containers—Well-closed containers.

Nicotinic Acid Injection

ニコチン酸注射液

Nicotinic Acid Injection is an aqueous injection.
It contains not less than 95.0% and not more than 110.0% of the labeled amount of nicotinic acid (C$_6$H$_5$NO$_2$: 123.11).

Method of preparation Prepare as directed under Injections, with Nicotinic Acid. It may contain Sodium Carbonate or Sodium Hydroxide as a solubilizer.

Description Nicotinic Acid Injection is a clear, colorless liquid.
pH: 5.0 – 7.0

Identification (1) To a volume of Nicotinic Acid Injection, equivalent to 0.1 g of Nicotinic Acid, add 0.3 mL of dilute hydrochloric acid, and evaporate on a water bath to 2 mL. After cooling, collect the crystals formed, wash with small portions of ice-cold water until the last washing shows no turbidity on the addition of silver nitrate TS, and dry at 105°C for 1 hour: the crystals melt *<2.60>* between 234°C and 238°C. With the crystals, proceed as directed in the Identification (1) under Nicotinic Acid.

(2) Dissolve 0.02 g of the dried crystals obtained in (1) in water to make 1000 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 261 nm and 263 nm, and a minimum between 235 nm and 239 nm. Separately, determine the absorbances of this solution, A_1 and A_2, at each wavelength of maximum and minimum absorption, respectively: the ratio A_2/A_1 is between 0.35 and 0.39.

Bacterial endotoxins *<4.01>* Less than 3.0 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 Measure exactly a volume of Nicotinic Acid Injection, equivalent to about 0.1 g of nicotinic acid (C$_6$H$_5$NO$_2$), and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 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 0.1 g of Nicotinic Acid RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add 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 nicotinic acid to that of the internal standard.

$$\text{Amount (mg) of nicotinic acid } (C_6H_5NO_2)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Nicotinic Acid RS taken

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).
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 35°C.
    Mobile phase: Dissolve 1.1 g of sodium 1-octane sulfonate in a mixture of 0.05 mol/L sodium dihydrogenphosphate TS (pH 3.0) and methanol (4:1) to make 1000 mL.
    Flow rate: Adjust so that the retention time of caffeine 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, nicotinic acid 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 ratios of the peak area of nicotinic acid to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Hermetic containers.

Nifedipine

ニフェジピン
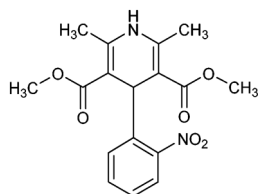
C$_{17}$H$_{18}$N$_2$O$_6$: 346.33
Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[*21829-25-4*]

Nifedipine contains not less than 98.0% and not more than 102.0% of nifedipine (C$_{17}$H$_{18}$N$_2$O$_6$), calculated on the dried basis.

Description   Nifedipine occurs as a yellow crystalline powder. It is odorless and tasteless.
    It is freely soluble in acetone and in dichloromethane, sparingly soluble in methanol, in ethanol (95) and in acetic acid (100), slightly soluble in diethyl ether, and practically insoluble in water.
    It is affected by light.

Identification   (1)   Dissolve 0.05 g of Nifedipine in 5 mL of ethanol (95), and add 5 mL of hydrochloric acid and 2 g of zinc powder. Allow to stand for 5 minutes, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for primary

aromatic amines, developing a red-purple color.
    (2)   Determine the absorption spectrum of a solution of Nifedipine 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 Nifedipine, 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*>   172 – 175°C

Purity   (1)   Clarity and color of solution—Dissolve 0.5 g of Nifedipine in 5 mL of acetone: the solution is clear and yellow.
    (2)   Chloride <*1.03*>—To 2.5 g of Nifedipine add 12 mL of dilute acetic acid and 13 mL of water, and heat to boil. After cooling, filter, and discard the first 10 mL of the filtrate. To 5 mL of the subsequent 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 0.021%).
    (3)   Sulfate <*1.14*>—To 4 mL of the filtrate obtained in (2) 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 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.054%).
    (4)   Heavy metals <*1.07*>—Proceed with 2.0 g of Nifedipine 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)   Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Nifedipine according to Method 3, and perform the test (not more than 2 ppm).
    (6)   Basic substances—The procedure should be performed under protection from light in light-resistant vessels. Dissolve 5.0 g of Nifedipine in 80 mL of a mixture of acetone and acetic acid (100) (5:3), and titrate <*2.50*> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction. Not more than 1.9 mL of 0.02 mol/L perchloric acid VS is consumed.
    (7)   Dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylate—The procedure should be performed under protection from light in light-resistant vessels. Dissolve 0.15 g of Nifedipine in dichloromethane to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine-dicarboxylate for thin-layer chromatography in exactly 10 mL of dichloromethane. Measure exactly 1 mL of this solution, add dichloromethane 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 cyclohexane and ethyl acetate (3:2) 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, corresponding to that from the standard solution, is 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,

2 hours).

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

Assay   The procedure should be performed under protection from light in light-resistant vessels. Weigh accurately about 0.12 g of Nifedipine, and dissolve in methanol to make exactly 200 mL. Measure exactly 5 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 350 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= A/142.3 \times 40,000$$

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

Nifedipine Delayed-release Fine Granules

ニフェジピン腸溶細粒

Nifedipine Delayed-release Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$: 346.33).

Method of preparation   Prepare as directed under Granules, with Nifedipine.

Identification   Conduct this procedure without exposure to light, using light-resistant vessels. Shake for 15 minutes a quantity of powdered Nifedipine Delayed-release Fine Granules, equivalent to 3 mg of Nifedipine, with 100 mL of methanol, and filter. Determine the absorption spectrum of the filtrate so obtained as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a broad absorption maximum between 335 nm and 356 nm.

Uniformity of dosage units <6.02>   Perform the test according to the following method: the Granules in single-dose packages meet the requirement of the Content uniformity test.

Conduct this procedure without exposure to light, using light-resistant vessels. To the total content of 1 package of Nifedipine Delayed-release Fine Granules add 50 mL of a mixture of methanol and water (9:1), agitate for 15 minutes with the aid of ultrasonic waves with occasional shaking, and shake for further 15 minutes. Then, add methanol to make exactly V mL so that each mL contains about 0.1 mg of nifedipine ($C_{17}H_{18}N_2O_6$). Filter this solution 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of nifedipine for assay taken

Dissolution <6.10>   When the tests are performed at 50 revolutions per minute according to the Paddle method, using 900 mL each of 1st and 2nd fluids for dissolution test as the dissolution medium, the dissolution rate in the test using the 1st fluid for dissolution test in 60 minutes is not more than 15%, and that in the test using the 2nd fluid for dissolution test in 30 minutes is not less than 75%.

Conduct this procedure without exposure to light, using light-resistant vessels. Start the test with an accurately weighed amount of Nifedipine Delayed-release Fine Granules, equivalent to about 20 mg of nifedipine ($C_{17}H_{18}N_2O_6$), 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 the dissolution medium to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nifedipine for assay, previously dried at 105°C for 2 hours, dissolve in 50 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium 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>, and determine the peak areas, A_T and A_S, of nifedipine in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount}$$
$$\text{of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= M_S/M_T \times A_T/A_S \times 1/C \times 72$$

M_S: Amount (mg) of nifedipine for assay taken
M_T: Amount (g) of Nifedipine Delayed-release Fine Granules taken
C: Labeled amount (mg) of nifedipine ($C_{17}H_{18}N_2O_6$) in 1 g

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 nifedipine are not less than 4000 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 nifedipine is not more than 1.0%.

Assay   Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately a portion of powdered Nifedipine Delayed-release Fine Granules, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), add 50 mL of a mixture of methanol and water (9:1), shake vigorously for 15 minutes, and add methanol to make exactly 100 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, pipet 5 mL of the subsequent filtrate, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol 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 nifedipine in each solution.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of nifedipine 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: Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.

Flow rate: Adjust so that the retention time of nifedipine 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 nifedipine are not less than 4000 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 nifedipine is not more than 1.0%.

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

Nifedipine Extended-release Capsules

ニフェジピン徐放カプセル

Nifedipine Extended-release Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$: 346.33).

Method of preparation   Prepare as directed under Capsules, with Nifedipine.

Identification   Conduct this procedure without exposure to light, using light-resistant vessels. Take out the content of Nifedipine Extended-release Capsules, and powder. To an amount of the powder, equivalent to 3 mg of Nifedipine, add 100 mL of methanol, shake for 15 minutes, and centrifuge. Determine the absorption spectrum of the supernatant liquid so obtained as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a broad absorption maximum between 335 nm and 356 nm.

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

Conduct this procedure without exposure to light, using light-resistant vessels. To the total content of 1 capsule of Nifedipine Extended-release Capsules, add 50 mL of a mixture of methanol and water (9:1), agitate for 15 minutes with the aid of ultrasonic waves with occasional shaking, and shake for further 15 minutes. Then, add methanol to make exactly V mL so that each mL contains about 0.1 mg of nifedipine ($C_{17}H_{18}N_2O_6$). Filter this solution 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at

105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of nifedipine for assay taken

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

Assay   Conduct this procedure without exposure to light, using light-resistant vessels. Take out the contents of not less than 20 Nifedipine Extended-release Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), add 50 mL of a mixture of methanol and water (9:1), shake vigorously for 15 minutes, and add methanol to make exactly 100 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, pipet 5 mL of the subsequent filtrate, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol 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 nifedipine in each solution.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of nifedipine 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: Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.

Flow rate: Adjust so that the retention time of nifedipine 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 nifedipine are not less than 4000 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 nifedipine is not more than 1.0%.

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

Nifedipine Fine Granules

ニフェジピン細粒

Nifedipine Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$: 346.33).

Method of preparation Prepare as directed under Granules, with Nifedipine.

Identification Conduct this procedure without exposure to light, using light-resistant vessels. Shake for 15 minutes a quantity of powdered Nifedipine Fine Granules, equivalent to 6 mg of Nifedipine, with 200 mL of methanol, and centrifuge. Determine the absorption spectrum of the supernatant liquid so obtained as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a broad absorption maximum between 335 nm and 356 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: the Granules in single-dose packages meet the requirement of the Content uniformity test.

Conduct this procedure without exposure to light, using light-resistant vessels. To the total content of 1 package of Nifedipine Fine Granules add 50 mL of a mixture of methanol and water (9:1), agitate for 15 minutes with the aid of ultrasonic waves with occasional shaking, and shake for further 15 minutes. Then, add methanol to make exactly V mL so that each mL contains about 0.1 mg of of nifedipine ($C_{17}H_{18}N_2O_6$). Filter this solution 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of nifedipine 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 Nifedipine Fine Granules is not less than 85%.

Conduct this procedure without exposure to light, using light-resistant vessels. Start the test with an accurately weighted amount of Nifedipine Fine Granules, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), 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 nifedipine for assay, previously dried at 105°C for 2 hours, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 2 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 nifedipine in each solution.

Dissolution rate (%) with respect to the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$)
$$= M_S/M_T \times A_T/A_S \times 1/C \times 36$$

M_S: Amount (mg) of nifedipine for assay taken
M_T: Amount (g) of Nifedipine Fine Granules taken
C: Labeled amount (mg) of nifedipine ($C_{17}H_{18}N_2O_6$) in 1 g

Operating conditions—
Proceed as directed in the operating conditions under 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 nifedipine are not less than 4000 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 nifedipine is not more than 1.0%.

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately a protein of powdered Nifedipine Fine Granules, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), add 50 mL of a mixture of methanol and water (9:1), shake vigorously for 15 minutes, and add methanol to make exactly 100 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, pipet 5 mL of the subsequent filtrate, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol 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 nifedipine in each solution.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6)$$
$$= M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of nifedipine 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: Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.
Flow rate: Adjust so that the retention time of nifedipine 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 nifedipine are not less than 4000 and not more than 1.2, respectively.
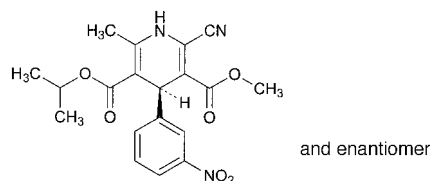System repeatability: When the test is repeated 6 times

with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nifedipine is not more than 1.0%.

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

Nilvadipine

ニルバジピン

and enantiomer

C$_{19}$H$_{19}$N$_3$O$_6$: 385.37
3-Methyl 5-(1-methylethyl) (4*RS*)-2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[*75530-68-6*]

Nilvadipine contains not less than 98.0% and not more than 102.0% of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$).

Description   Nilvadipine occurs as a yellow crystalline powder.

It is freely soluble in acetonitrile, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Nilvadipine in acetonitrile (1 in 20) shows no optical rotation.

Identification   **(1)**   Determine the absorption spectrum of a solution of Nilvadipine 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 Nilvadipine 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 Nilvadipine 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 Nilvadipine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60>   167 – 171°C

Purity   **(1)**   Heavy metals <1.07>—Proceed with 2.0 g of Nilvadipine 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 Nilvadipine in 20 mL of acetonitrile, 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 each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each related substance is not more than 0.3%, and the total of them is not more than 0.5%.
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 25°C.
  Mobile phase: A mixture of phosphate buffer solution (pH 7.4), methanol and acetonitrile (32:27:18).
  Flow rate: Adjust so that the retention time of nilvadipine is about 12 minutes.
  Time span of measurement: About 2.5 times as long as the retention time of nilvadipine, beginning after the solvent peak.
System suitability—
  Test for required detectability: To 1 mL of the sample solution, add acetonitrile 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 acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine 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: When the procedure is run with 5 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 nilvadipine is not less than 3300 and not more than 1.3, respectively.
  System repeatability: Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

Loss on drying <2.41>   Not more than 0.1% (1 g, 105°C, 2 hours).

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

Assay   Weigh accurately about 25 mg each of Nilvadipine and Nilvadipine RS, dissolve in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 20 mL of the internal standard solution, 20 mL of water and methanol to make 100 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 the Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of nilvadipine to that of the internal standard.

Amount (mg) of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Nilvadipine RS taken

*Internal standard solution—*A solution of acenaphthene in methanol (1 in 200).
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 25°C.
  Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.
  Flow rate: Adjust so that the retention time of nilvadipine is about 12 minutes.
System suitability—
  System performance: When the procedure is run with 5 μL

of the standard solution under the above operating conditions, nilvadipine 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 nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Nilvadipine Tablets

ニルバジピン錠

Nilvadipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$: 385.37).

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

Identification   To a quantity of powdered Nilvadipine Tablets, equivalent to 1 mg of Nilvadipine, add 100 mL of ethanol (99.5), shake for 10 minutes, centrifuge, and use the supernatant liquid 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 239 nm and 243 nm and a maximum having a broad-ranging absorption between 371 nm and 381 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 Nilvadipine Tablets add V mL of a mixture of acetonitrile and water (7:3) so that each mL of the solution contains about 0.2 mg of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$), add exactly V mL of the internal standard solution, and disperse the particles with the aid of ultrasonic waves. Centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$)
= $M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of Nilvadipine RS taken

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 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 30 minutes of Nilvadipine Tablets is not less than 85%.

Start the test with 1 tablet of Nilvadipine 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 10 mL of the subsequent filtrate, add exactly 1 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately an amount of

Nilvadipine RS, equivalent to 10 times the labeled amount of Nilvadipine Tablets, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of water, 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 nilvadipine in each solution.

Dissolution rate (%) with respect to the labeled amount of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$)
= $M_S \times A_T/A_S \times 1/C \times 9$

M_S: Amount (mg) of Nilvadipine RS taken
C: Labeled amount (mg) of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 242 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.
Mobile phase: A mixture of phosphate buffer solution (pH 7.4), methanol and acetonitrile (7:7:6).
Flow rate: Adjust so that the retention time of nilvadipine 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 nilvadipine 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 peak area of nilvadipine is not more than 1.5%.

Assay   Weigh accurately not less than 20 Nilvadipine Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 5 mg of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$), add 10 mL of a mixture of acetonitrile and water (7:3) and exactly 25 mL of the internal standard solution, shake for 15 minutes, and add the mixture of acetonitrile and water (7:3) to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine RS, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) 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 nilvadipine to that of the internal standard.

Amount (mg) of nilvadipine (C$_{19}$H$_{19}$N$_3$O$_6$)
= $M_S \times Q_T/Q_S \times 1/4$

M_S: Amount (mg) of Nilvadipine RS taken

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wave-

length: 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 25°C.

Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.

Flow rate: Adjust so that the retention time of nilvadipine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine 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 nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Nitrazepam

ニトラゼパム
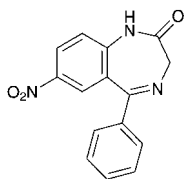
$C_{15}H_{11}N_3O_3$: 281.27
7-Nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*146-22-5*]

Nitrazepam, when dried, contains not less than 99.0% of nitrazepam ($C_{15}H_{11}N_3O_3$).

Description   Nitrazepam occurs as white to yellow, crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in acetone and in chloroform, slightly soluble in methanol, in ethanol (95) and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 227°C (with decomposition).

Identification   **(1)** To 3 mL of a solution of Nitrazepam in methanol (1 in 500) add 0.1 mL of sodium hydroxide TS: a yellow color is produced.

(2) To 0.02 g of Nitrazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(3) Neutralize 0.5 mL of the filtrate obtained in (2) with sodium hydroxide TS, add 2 mL of ninhydrin TS, and heat on a water bath: a purple color is produced.

(4) Determine the absorption spectrum of a solution of Nitrazepam 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.

Purity   **(1)** Clarity and color of solution—Dissolve 0.10 g of Nitrazepam in 20 mL of acetone: the solution is clear and pale yellow to light yellow in color.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Nitrazepam 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 Nitrazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Nitrazepam in a 10 mL of mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, pipet 2 mL of this solution, add a mixture of methanol and chloroform (1:1) 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 nitromethane and ethyl acetate (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 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 Nitrazepam, previously dried, and dissolve in 40 mL of acetic acid (100). 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
= 28.13 mg of $C_{15}H_{11}N_3O_3$

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

Nitrendipine

ニトレンジピン

and enantiomer
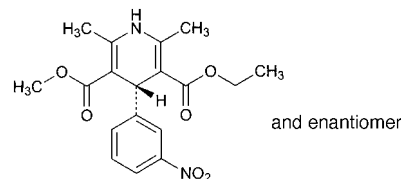
$C_{18}H_{20}N_2O_6$: 360.36
3-Ethyl 5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-
1,4-dihydropyridine-3,5-dicarboxylate
[*39562-70-4*]

Nitrendipine, when dried, contains not less than 98.5% and not more than 101.0% of nitrendipine ($C_{18}H_{20}N_2O_6$).

Description   Nitrendipine occurs as a yellow crystalline

powder.

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

It is gradually colored to brownish yellow by light.

A solution of Nitrendipine in acetonitrile (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Nitrendipine in methanol (1 in 80,000) 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 Nitrendipine 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> 157 – 161°C

Purity **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Nitrendipine 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—Conduct this procedure rapidly using light-resistant vessels. Dissolve 40 mg of Nitrendipine in 5 mL of acetonitrile, add the mobile phase to make 25 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 immediately 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, and calculate the amount of related substances by the following equation: the amount of a related substance, having the relative retention time of about 0.8 to nitrendipine, is not more than 1.0%, a related substance, having the relative retention time of about 1.3, is not more than 0.25%, and other related substances are not more than 0.2%, respectively. The total amount of the related substances other than nitrendipine is not more than 2.0%.

$$\text{Amount (\%) of related substance} = A_T/A_S$$

A_T: Each peak area other than nitrendipine obtained from the sample solution

A_S: Peak area of nitrendipine obtained from the standard solution

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, tetrahydrofuran and acetonitrile (14:6:5).

Flow rate: Adjust so that the retention time of nitrendipine is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of nitrendipine, 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 10 mL. Confirm that the peak area of nitrendipine obtained with 10 μL of this solution is equivalent to 14 to 26% of that obtained with 10 μL of the standard solution.

System performance: Dissolve 10 mg of Nitrendipine and 3 mg of propyl parahydroxybenzoate in 5 mL of acetonitrile, and add the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and nitrendipine 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 peak area of nitrendipine 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 Nitrendipine, previously dried, dissolve in 60 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 100), add 50 mL of water, and titrate <2.50> with 0.1 mol/L serium (IV) tetraammonium sulfate VS until the red-orange color of the solution vanishes (indicator: 3 drops of 1,10-phenanthroline TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L serium (IV) tetraammonium sulfate VS
   = 18.02 mg of $C_{18}H_{20}N_2O_6$

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

Nitrendipine Tablets

ニトレンジピン錠

Nitrendipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$: 360.36).

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

Identification Shake a quantity of powdered Nitrendipine Tablets, equivalent to 5 mg of Nitrendipine, with 70 mL of methanol, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 234 nm and 238 nm, and between 350 nm and 354 nm.

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

Conduct this procedure using light-resistant vessels. To 1 tablet of Nitrendipine Tablets add 15 mL of diluted acetonitrile (4 in 5), stir until the tablet is completely disintegrated, and further stir for 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 20 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 1 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (4 in 5) to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of nitrendipine ($C_{18}H_{20}N_2O_6$)
= $M_S \times Q_T/Q_S \times 1/V \times 1/5$

M_S: Amount (mg) of nitrendipine for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

Dissolution <*6.10*>   When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium containing 3 g of polysorbate 80 in 5 L of water for 5-mg tablet and the dissolution medium containing 3 g of polysorbate 80 in 2000 mL of water for 10-mg tablet, the dissolution rate in 45 minutes of Nitrendipine Tablets is not less than 70%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Nitrendipine 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 the subsequent V mL, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of nitrendipine ($C_{18}H_{20}N_2O_6$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nitrendipine for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium 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, A_T and A_S, of nitrendipine in each solution.

Dissolution rate (%) with respect to the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of nitrendipine for assay taken
C: Labeled amount (mg) of nitrendipine ($C_{18}H_{20}N_2O_6$) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 356 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, tetrahydrofuran and acetonitrile (14:6:5).
Flow rate: Adjust so that the retention time of nitrendipine 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 nitrendipine 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 nitrendipine is not more than 2.0%.

Assay   Conduct this procedure using light-resistant vessels. To 20 tablets of Nitrendipine Tablets add 150 mL of diluted acetonitrile (4 in 5), stir until the tablets completely disintegrate, and stir for further 10 minutes. Add diluted aceto-

nitrile (4 in 5) to make exactly 200 mL, and centrifuge. Pipet a volume of the supernatant liquid, equivalent to about 2 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 10 mL of the internal standard solution and diluted acetonitrile (4 in 5) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nitrendipine for assay, previously dried at 105°C for 2 hours, and dissolve in diluted acetonitrile (4 in 5) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution and diluted acetonitrile (4 in 5) 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 nitrendipine to that of the internal standard.

Amount (mg) of nitrendipine ($C_{18}H_{20}N_2O_6$)
= $M_S \times Q_T/Q_S \times 1/50$

M_S: Amount (mg) of nitrendipine for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).
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, tetrahydrofuran and acetonitrile (14:6:5).
Flow rate: Adjust so that the retention time of nitrendipine 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, the internal standard and nitrendipine 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 peak area of nitrendipine is not more than 1.0%.

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

Nitrogen

窒素

N$_2$: 28.01

Nitrogen is the nitrogen produced by the air liquefaction separation method.
It contains not less than 99.5 vol% of nitrogen (N$_2$).

Description   Nitrogen is a colorless gas at room temperature and under atmospheric pressure, and is odorless.
1 mL of Nitrogen dissolves in 65 mL of water and in 9 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.
1000 mL of Nitrogen at 0°C and at a pressure of 101.3 kPa weighs 1.251 g.

Identification   Introduce 1 mL each of Nitrogen and nitro-

gen into a gas-measuring tube or syringe for gas chromatography from a cylinder with a pressure-reducing valve, through a directly connected polyvinyl chloride or stainless steel tube. Perform the test with these gases as directed under Gas Chromatography <2.02> according to the following conditions: the principal peak in the chromatogram obtained form Nitrogen has the same retention time as that obtained from nitrogen.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

Purity Oxygen—The peak area of oxygen obtained from Nitrogen in the Assay is not larger than 1/2 times that obtained from the standard gas mixture.

Assay Introduce 1.0 mL of Nitrogen into a gas-measuring tube or syringe for gas chromatography from a cylinder with a pressure-reducing valve, through a directly connected polyvinyl chloride or stainless steel tube. Perform the test with this gas as directed under Gas Chromatography <2.02> according to the following conditions. Measure the peak area A_T of oxygen. Separately, introduce 1.0 mL of oxygen into the gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard gas mixture. Proceed with 1.0 mL of this mixture in the same manner under Nitrogen, and measure the peak area A_S of oxygen.

$$\text{Amount (vol\%) of nitrogen (N}_2) = 100 - A_T/A_S$$

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with zeolite for gas chromatography (250 to 355 μm in particle diameter; 0.5 nm in pore size).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust so that the retention time of oxygen is about 3 minutes.

System suitability—

System performance: Introduce 1.0 mL of oxygen into the gas mixer, add Nitrogen to make 100 mL, and mix thoroughly. When the procedure is run with 1.0 mL of this mixture under the above operating conditions, oxygen and nitrogen are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 1.0 mL of the standard gas mixture under the above conditions, the relative standard deviation of the peak area of oxygen is not more than 2.0%.

Containers and storage Containers—Pressure-resistant cylinders.

Storage—Not exceeding 40°C.

Nitroglycerin Tablets

ニトログリセリン錠

Nitroglycerin Tablets contain not less than 80.0% and not more than 120.0% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$: 227.09).

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

Identification (1) Weigh a quantity of powdered Nitroglycerin Tablets, equivalent to 6 mg of nitroglycerin

($C_3H_5N_3O_9$), shake thoroughly with 12 mL of diethyl ether, filter, and use the filtrate as the sample solution. Evaporate 5 mL of the sample solution, dissolve the residue in 1 to 2 drops of sulfuric acid, and add 1 drop of diphenylamine TS: a deep blue color develops.

(2) Evaporate 5 mL of the sample solution obtained in (1), add 5 drops of sodium hydroxide TS, heat over a low flame, and concentrate to about 0.1 mL. Cool, heat the residue with 0.02 g of potassium hydrogen sulfate: the odor of acrolein is perceptible.

Purity Free nitrate ion—Transfer an accurately measured quantity of powdered Nitroglycerin Tablets, equivalent to 20 mg of nitroglycerin ($C_3H_5N_3O_9$), to a separator, add 40 mL of isopropylether and 40 mL of water, shake for 10 minutes, and allow the layers to separate. Collect the aqueous layer, add 40 mL of isopropylether, shake for 10 minutes, collect the aqueous layer, filter, and use the filtrate as the sample solution. Separately, transfer 10 mL of Standard Nitric Acid Solution to a separator, add 30 mL of water and 40 mL of the isopropyl ether layer of the first extraction of the sample solution, shake for 10 minutes, continue the procedure in the same manner as the sample solution, and use the solution so obtained as the standard solution. Transfer 20 mL each of the sample solution and the standard solution to Nessler tubes, respectively, shake well with 30 mL of water and 0.06 g of Griess-Romijin's nitric acid reagent, allow to stand for 30 minutes, and observe the tubes horizontally: the sample solution has no more color than 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.

Transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, and add exactly V mL of acetic acid (100) to provide a solution containing about 30 μg of nitroglycerin ($C_3H_5N_3O_9$) per ml. Shake vigorously for 1 hour, and after disintegrating the tablet, centrifuge, and use the supernatant liquid as the sample solution. When the tablet does not disintegrate during this procedure, transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, wet the tablet with 0.05 mL of acetic acid (100), and grind down it with a glass rod. While rinsing the glass rod, add acetic acid (100) to make exactly V mL of a solution containing about 30 μg of nitroglycerin ($C_3H_5N_3O_9$) per ml. Shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 5 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, add 2 mL each of salicylic acid TS shake, allow to stand for 15 minutes, and add 10 mL each of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2 mL of acetic acid (100) 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 410 nm, respectively.

$$\text{Amount (mg) of nitroglycerin (C}_3H_5N_3O_9)$$
$$= M_S \times A_T/A_S \times V/2000 \times 0.749$$

M_S: Amount (mg) of potassium nitrate taken

Calculate the average content from the contents of 10 tablets: it meets the requirements of the test when each content deviates from the average content by not more than 25%. When there is 1 tablet showing a deviation exceeding 25% and not exceeding 30%, determine the content of an additional 20 tablets in the same manner. Calculate the 30 deviations from the new average of all 30 tablets: it meets the requirements of the test when 1 tablet may deviate from the average content by between 25% and 30%, but no tablet deviates by more than 30%.

Disintegration *⟨6.09⟩*   It meets the requirement, provided that the time limit of the test is 2 minutes, and the use of the disks is omitted.

Assay   Weigh accurately and disintegrate, by soft pressing, not less than 20 Nitroglycerin Tablets. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of nitroglycerin ($C_3H_5N_3O_9$), add exactly 50 mL of acetic acid (100), shake for 1 hour, filter, and use this filtrate as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 10 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, to each solution add 2 mL of salicylic acid TS, shake, allow to stand for 15 minutes, and add 10 mL of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, using a solution, prepared with 2 mL of acetic acid (100) 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 410 nm, respectively.

$$\text{Amount (mg) of nitroglycerin } (C_3H_5N_3O_9)$$
$$= M_S \times A_T/A_S \times 1/20 \times 0.749$$

M_S: Amount (mg) of potassium nitrate taken

Containers and storage   Containers—Tight containers.
Storage—Light-resistant, and not exceeding 20°C.

Nitrous Oxide

亜酸化窒素

N_2O: 44.01

Nitrous Oxide contains not less than 97.0 vol% of nitrous oxide (N_2O).

Description   Nitrous Oxide is a colorless gas at room temperature and at atmospheric pressure, and is odorless.

1 mL of Nitrous Oxide dissolves in 1.5 mL of water and in 0.4 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa. It is soluble in diethyl ether and in fatty oils.

1000 mL of Nitrous Oxide at 0°C and at a pressure of 101.3 kPa weighs about 1.96 g.

Identification   (1)   A glowing splinter of wood held in Nitrous Oxide: it bursts into flame immediately.

(2)   Transfer 1 mL each of Nitrous Oxide and nitrous oxide directly from metal cylinders with a pressure-reducing valve to gas measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography ⟨2.02⟩ according to the operating conditions of the Assay: the retention time of the main peak in the chromatogram obtained with Nitrous Oxide coincides with that in the chromatogram obtained with nitrous oxide.

Purity   Maintain the containers of Nitrous Oxide between 18°C and 22°C for more than 6 hours before the test, and correct the volume at 20°C and at a pressure of 101.3 kPa.

(1)   Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 1000 mL of Nitrous Oxide through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

(2)   Carbon dioxide—Pass 1000 mL of Nitrous Oxide through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

(3)   Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 2000 mL of Nitrous Oxide through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

(4)   Potassium permanganate-reducing substance—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.10 mL of 0.02 mol/L potassium permanganate VS to each of the tubes, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the manner as directed in (1): the color of solution A is the same as that of solution B.

(5)   Chloride—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

(6)   Carbon monoxide—Introduce 5.0 mL of Nitrous Oxide into a gas-cylinder or a syringe for gas chromatography from a metal cylinder holding gas under pressure and fitted with a pressure-reducing valve, through a directly connected polyvinyl tube. Perform the test with this under Gas Chromatography ⟨2.02⟩ according to the following conditions: no peak is observed at the same retention time as that of carbon monoxide.
Operating conditions—
   Detector: A thermal conductivity detector.
   Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 300 to 500 μm zeolite for gas chromatography (0.5 nm in pore size).
   Column temperature: A constant temperature of about

50°C.

Carrier gas: Hydrogen or helium.

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

Selection of column: To 0.1 mL each of carbon monoxide and air in a gas mixer add carrier gas to make 100 mL, and mix well. Proceed with 5.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of oxygen, nitrogen and carbon monoxide in this order.

Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the selection of column is about 10 cm.

Assay Withdraw Nitrous Oxide as directed in the Purity.

Introduce 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test with this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A_T of air. Separately, introduce 3.0 mL of nitrogen into a gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard mixed gas. Proceed with 1.0 mL of this mixture as directed in the case of Nitrous Oxide, and determine the peak area A_S of nitrogen in the same manner.

Amount (vol%) of nitrous oxide (N$_2$O) = $100 - 3 \times A_T/A_S$

Operating conditions—

Detector: A thermal conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with silica gel for gas chromatography (300 to 500 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust so that the retention time of nitrogen is about 2 minutes.

Selection of column: To 3.0 mL of nitrogen in a gas mixer add Nitrous Oxide to make 100 mL, and mix well. Proceed with 1.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and nitrous oxide in this order.
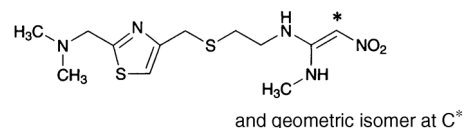
System repeatability: Repeat the test five times with the standard mixed gas under the above operating conditions: the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

Containers and storage Containers—Metal cylinders.

Storage—Not exceeding 40°C.

Nizatidine

ニザチジン

and geometric isomer at C*

C$_{12}$H$_{21}$N$_5$O$_2$S$_2$: 331.46
(1*EZ*)-*N*-{2-[({2-[(Dimethylamino)methyl]thiazol-4-yl}methyl)sulfanyl]ethyl}-*N*′-methyl-2-nitroethene-1,1-diamine
[*76963-41-2*]

Nizatidine, when dried, contains not less than 98.0% and not more than 101.0% of nizatidine (C$_{12}$H$_{21}$N$_5$O$_2$S$_2$).

Description Nizatidine occurs as a white to pale yellowish white crystalline powder, and has a characteristic odor.

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

Identification (1) Determine the absorption spectrum of a solution of Nizatidine 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 Nizatidine 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 Nizatidine, 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 Nizatidine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 130 – 135°C (after drying).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nizatidine according to Method 4, and perform the test using 3 mL of sulfuric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Nizatidine in 10 mL of a mixture of the mobile phase A and mobile phase B (19:6), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of the mobile phase A and mobile phase B (19:6) 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. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than nizatidine obtained from the sample solution is not larger than 1/5 times the nizatidine obtained from the standard solution. Furthermore, the total of the areas of peaks other than the nizatidine from the sample solution is not larger than the peak area of nizatidine 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 A: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100).

Mobile phase B: Methanol.

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	76	24
3 – 20	76 → 50	24 → 50
20 – 45	50	50

Flow rate: 1.0 mL per minute.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of the mobile phase A and mobile phase B (19:6) to make exactly 25 mL. Confirm that the peak area of nizatidine obtained from 50 μL of this solution is equivalent to 15 to 25% 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 nizatidine are not less than 20,000 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 nizatidine is not more than 2.0%.

Loss on drying *<2.41>*   Not more than 0.5% (2 g, 100°C, 1 hour).

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

Assay   Weigh accurately about 15 mg each of Nizatidine and Nizatidine RS, both previously dried, dissolve each 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 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 area, A_T and A_S, of nizatidine in each solution.

Amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Nizatidine RS taken

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 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.

Flow rate: Adjust so that the retention time of nizatidine 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 number of theoretical plates and the symmetry factor of the peak of nizatidine 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 nizatidine is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Nizatidine Capsules

ニザチジンカプセル

Nizatidine Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$: 331.46).

Method of preparation   Prepare as directed under Capsules, with Nizatidine.

Identification   Take out the contents of Nizatidine Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Nizatidine, add 50 mL of methanol, shake well, and filter. Pipet 1 mL of the filtrate, 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 239 nm and 244 nm, and between 323 nm and 327 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.

Take out the contents from 1 capsule of Nizatidine Capsules, add the mobile phase to make exactly V mL so that each mL contains about 1.5 mg of nizatidine ($C_{12}H_{21}N_5O_2S_2$). After shaking vigorously for 10 minutes, centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution and add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
  = $M_S \times Q_T/Q_S \times V/10$

M_S: Amount (mg) of Nizatidine RS taken

*Internal standard solution—*A solution of phenol in the mobile phase (1 in 100).

Dissolution *<6.10>*   When the test is performed at 50 revolutions per minute according to the Paddle method using a sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Nizatidine Capsules is not less than 80%.

Start the test with 1 capsule of Nizatidine Capsules, 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 2 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 10 μg of nizatidine ($C_{12}H_{21}N_5O_2S_2$). Use this solution as the sample solution. Separately, weigh accurately about 25 mg of Nizatidine RS, previously dried at 100°C for 1 hour, and dissolve in water to make exactly 100 mL. Pipet

2 mL of this solution, 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 Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 314 nm.

Dissolution rate (%) with respect to the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
 = $M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Nizatidine RS taken
C: Labeled amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$) in 1 capsule

Assay   Take out the contents of not less than 10 Nizatidine Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of nizatidine ($C_{12}H_{21}N_5O_2S_2$), add exactly 50 mL of the mobile phase, shake vigorously for 10 minutes, 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 15 mg of Nizatidine RS, previously dried at 100°C for 1 hour, dissolve in 30 mL of the mobile phase, 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 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 nizatidine to that of the internal standard.

Amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
 = $M_S \times Q_T/Q_S \times 10$

M_S: Amount (mg) of Nizatidine RS taken

Internal standard solution—A solution of phenol 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 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 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.
 Flow rate: Adjust so that the retention time of nizatidine 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 nizatidine are eluted in this order with the resolution between these peaks being not less than 3.
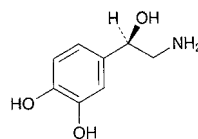 System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nizatidine to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Noradrenaline

Norepinephrine

ノルアドレナリン

and enantiomer

$C_8H_{11}NO_3$: 169.18
4-[(1*RS*)-2-Amino-1-hydroxyethyl]benzene-1,2-diol
[*51-41-2*]

Noradrenaline, when dried, contains not less than 98.0% of *dl*-noradrenaline ($C_8H_{11}NO_3$).

Description   Noradrenaline occurs as a white to light brown or slightly reddish brown crystalline powder.
 It is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in ethanol (95).
 It dissolves in dilute hydrochloric acid.
 It gradually changes to brown by air and by light.

Identification   **(1)**   Determine the absorption spectrum of a solution of Noradrenaline in 0.1 mol/L hydrochloric acid TS (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 Noradrenaline, 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 0.10 g of Noradrenaline in 10 mL of 0.1 mol/L hydrochloric acid TS, and add water to make 100 mL: the solution is clear and colorless.
 (2)   Arterenone—Dissolve 50 mg of Noradrenaline in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.1.
 (3)   Adrenaline—Dissolve 10.0 mg of Noradrenaline in 2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of this solution, add water to make 10 mL, then mix with 0.3 mL of a solution of sodium nitrite (1 in 100), and allow to stand for 1 minute: the solution has no more color than the following control solution.
 Control solution: Dissolve 2.0 mg of Adrenaline Bitartrate RS and 90 mg of Noradrenaline Bitartrate RS in water to make exactly 10 mL. Measure exactly 1 mL of this solution, add 1.0 mL of diluted acetic acid (100) (1 in 2) and water to make 10 mL, and proceed in the same manner.

Loss on drying <2.41>   Not more than 1.0% (1 g, in vacuum, silica gel, 18 hours).

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

Assay   Weigh accurately about 0.3 g of Noradrenaline, previously dried, dissolve in 50 mL of acetic acid for nonaqueous titration by warming, if necessary, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solu-

tion changes from blue-purple through blue to blue-green (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
= 16.92 mg of $C_8H_{11}NO_3$

Containers and storage   Containers—Tight containers.
   Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Noradrenaline Injection

Noradrenaline Hydrochloride Injection
Norepinephrine Hydrochloride Injection
Norepinephrine Injection

ノルアドレナリン注射液

Noradrenaline Injection is an aqueous injection.
   It contains not less than 90.0% and not more than 110.0% of the labeled amount of *dl*-noradrenaline ($C_8H_{11}NO_3$: 169.18).

Method of preparation   Dissolve Noradrenaline in 0.01 mol/L hydrochloric acid TS, and prepare as directed under Injections.

Description   Norepinephrine Injection is a clear, colorless liquid.
   It gradually becomes a pale red color by light and by air.
   pH: 2.3 – 5.0

Identification   Transfer a volume of Noradrenaline Injection, equivalent to 1 mg of Noradrenaline, to each of two test tubes A and B, and add 1 mL of water to each tube. Add 10 mL of potassium hydrogen phthalate buffer solution (pH 3.5) to A, and 10 mL of phosphate buffer solution (pH 6.5) to B. To each of these solutions add 1.0 mL of iodine TS, allow to stand for 5 minutes, and add 2.0 mL of sodium thiosulfate TS: no color or a pale red color develops in test tube A, and a deep red-purple color develops in test tube B.

Purity   **(1)**   Arterenone—Measure a volume of Noradrenaline Injection, equivalent to 10 mg of Noradrenaline, add water to make exactly 20 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance is not more than 0.10.
   (2)   Adrenaline—Measure a volume of Noradrenaline Injection, equivalent to 5 mg of Noradrenaline, add 1 mL of diluted acetic acid (100) (1 in 2) and water to make exactly 10 mL, and proceed as directed in the Purity (3) under Noradrenaline.

Bacterial endotoxins <4.01>   Less than 300 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 Noradrenaline Injection, equivalent to about 5 mg of *dl*-noradrenaline ($C_8H_{11}NO_3$), add

water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Noradrenaline Bitartrate RS, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add 0.2 mL each of starch TS, then add iodine TS dropwise with swirling until a persistent blue color is produced. Add 2 mL of iodine TS, and shake. Adjust the pH of the solution to 6.5 with 0.05 mol/L disodium hydrogenphosphate TS, add 10 mL of phosphate buffer solution (pH 6.5), and shake. Immediately after allowing to stand for 3 minutes, add sodium thiosulfate TS dropwise until a red-purple color develops, then add water to make exactly 50 mL. Determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 515 nm within 5 minutes as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of *dl*-noradrenaline ($C_8H_{11}NO_3$)
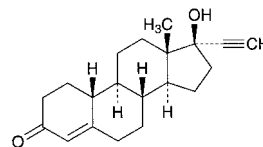= $M_S \times A_T/A_S \times 0.502$

M_S: Amount (mg) of Noradrenaline Bitartrate RS taken

Containers and storage   Containers—Hermetic containers, and colored containers may be used.
   Storage—Light-resistant.

Norethisterone

ノルエチステロン

$C_{20}H_{26}O_2$: 298.42
17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one
[*68-22-4*]

Norethisterone, when dried, contains not less than 97.0% and not more than 103.0% of norethisterone ($C_{20}H_{26}O_2$).

Description   Norethisterone occurs as a white to pale yellowish white crystalline powder. It has no odor.
   It is sparingly soluble in ethanol (95), in acetone, and in tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.
   It is affected by light.

Identification   **(1)**   To 2 mg of Norethisterone add 2 mL of sulfuric acid: the solution shows a red-brown color and a yellow-green fluorescence. Add 10 mL of water to this solution cautiously: a yellow color develops and a yellow-brown precipitate is formed.
   (2)   Determine the infrared absorption spectrum of Norethisterone 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> $[\alpha]_D^{20}$: -32 – $-37°$ (after drying, 0.25 g, acetone, 25 mL, 100 mm).

Melting point <2.60>   203 – 209°C

Loss on drying ⟨2.41⟩   Not more than 0.5% (0.5 g, in vacuum, silica gel, 4 hours).

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

Assay   Weigh accurately about 0.2 g of Norethisterone, previously dried, 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). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L sodium hydroxide VS}$$
$$= 29.84 \text{ mg of } C_{20}H_{26}O_2$$

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

Norfloxacin

ノルフロキサシン
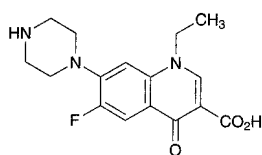
$C_{16}H_{18}FN_3O_3$: 319.33
1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-
1,4-dihydroquinoline-3-carboxylic acid
[70458-96-7]

Norfloxacin, when dried, contains not less than 99.0% of norfloxacin ($C_{16}H_{18}FN_3O_3$).

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

It is freely soluble in acetic acid (100), slightly soluble in ethanol (99.5) and in acetone, very slightly soluble in methanol, and practically insoluble in water.

It dissolves in dilute hydrochloric acid TS and in sodium hydroxide TS.

It is hygroscopic.

It is gradually colored by light.

Identification   (1)   Dissolve 0.01 g of Norfloxacin in a solution of sodium hydroxide (1 in 250) to make 100 mL. To 5 mL of this solution add a solution of sodium hydroxide (1 in 250) 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)   Dissolve a suitable amount of Norfloxacin in a suitable amount of acetone, evaporate the acetone under reduced pressure, and dry the residue. 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)   Sulfate ⟨1.14⟩—Dissolve 1.0 g of Norfloxacin in 7 mL of 0.5 mol/L sodium hydroxide TS and 23 mL of water, and add 1 drop of phenolphthalein TS. Add gradually diluted hydrochloric acid (1 in 3) to this solution until the red color disappears, then add 0.5 mL of dilute hydrochloric acid, and cool in ice for 30 minutes. Filter through a glass filter (G4), and wash the residue with 10 mL of water. Combine the filtrate and the washing, 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 0.50 mL of 0.005 mol/L sulfuric acid VS add 7 mL of 0.5 mol/L sodium hydroxide TS and 1 drop of phenolphthalein TS, add diluted hydrochloric acid (1 in 3) until the red color disappears, then add 1.5 mL of dilute hydrochloric acid, 1 or 2 drops of bromophenol blue TS and water to make 50 mL (not more than 0.024%).

(2)   Heavy metals ⟨1.07⟩—Proceed with 2.0 g of Norfloxacin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

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

(4)   Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Norfloxacin in 50 mL of a mixture of methanol and acetone (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetone (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of methanol and acetone (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 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5 to 7 μm in particle diameter). Develop with a mixture of methanol, chloroform, toluene, diethylamine and water (20:20:10:7:4) to a distance of about 9 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the number of the spot 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 1.0% (1 g, 105°C, 2 hours).

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

Assay   Weigh accurately about 0.5 g of Norfloxacin, 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.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 31.93 \text{ mg of } C_{16}H_{18}FN_3O_3$$

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

Norgestrel

ノルゲストレル
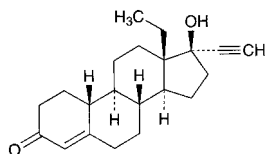
C$_{21}$H$_{28}$O$_2$: 312.45
13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one
[*6533-00-2*]

Norgestrel, when dried, contains not less than 98.0% of norgestrel (C$_{21}$H$_{28}$O$_2$).

Description   Norgestrel occurs as white, crystals or crystalline powder.

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

Identification   (1)   Dissolve 1 mg of Norgestrel in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. With this solution, examine under ultraviolet light (main wavelength: 365 nm): the solution shows a red-orange fluorescence.

(2)   Determine the infrared absorption spectrum of Norgestrel, 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>   206 – 212°C

Purity   (1)   Heavy metals <1.07>—Take 1.0 g of Norgestrel, heat gently to carbonize, cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution 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 30 mg of Norgestrel in 5 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and ethyl acetate (2:1) 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% (1 g, 105°C, 3 hours).

Residue on ignition <2.44>   Not more than 0.2% (0.5 g).

Assay   Weigh accurately about 0.2 g of Norgestrel, previously dried, 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 titra-

tion). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 31.25 mg of C$_{21}$H$_{28}$O$_2$

Containers and storage   Containers—Well-closed containers.

Norgestrel and Ethinylestradiol Tablets

ノルゲストレル・エチニルエストラジオール錠

Norgestrel and Ethinylestradiol Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of norgestrel (C$_{21}$H$_{28}$O$_2$: 312.45) and ethinylestradiol (C$_{20}$H$_{24}$O$_2$: 296.40).

Method of preparation   Prepare as directed under Tablets, with Norgestrel and Ethinylestradiol.

Identification   (1)   Weigh a quantity of Norgestrel and Ethinylestradiol Tablets, equivalent to 10 mg of Norgestrel, previously powdered, add 10 mL of chloroform, shake for 10 minutes, and filter. To 2 mL of the filtrate add 6 mL of sodium hydroxide TS, shake vigorously, and centrifuge. Take 1 mL of the chloroform layer, evaporate on a water bath to dryness, dissolve the residue in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. Examine under ultraviolet light (main wavelength: 365 nm): this solution shows a red-orange fluorescence (norgestrel).

(2)   Take 1 mL of the filtrate obtained in (1), evaporate on a water bath to dryness, add 1 mL of boric acid-methanol buffer solution to the residue, shake, and cool in ice. Add 1 mL of ice-cold diazo TS, shake, add 1 mL of sodium hydroxide TS, and shake: a red-orange color develops (ethinylestradiol).

(3)   Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 10 mg of Norgestrel RS and 1 mg of Ethinylestradiol RS, respectively, in 10 mL of chloroform, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 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 1,2-dichloroethane, methanol and water (368:32:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of *p*-toluenesulfonate monohydrate in ethanol (95) (1 in 5) on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365nm): two spots from the sample solution show the similar color tone and *R*f value to each spot from the standard solutions (1) and (2).

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

Add 2 mL of diluted methanol (7 in 10) to 1 tablet of Norgestrel and Ethinylestradiol Tablets, add exactly 2 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately quantities of Norgestrel RS and of Ethinylestradiol RS, equivalent to 100 times each of the labeled amounts, dissolve in diluted methanol (7 in 10) to make exactly 200 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 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} and Q_{Tb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

$$\text{Amount (mg) of norgestrel (C}_{21}\text{H}_{28}\text{O}_2)$$
$$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/100$$

$$\text{Amount (mg) of ethinylestradiol (C}_{20}\text{H}_{24}\text{O}_2)$$
$$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/100$$

M_{Sa}: Amount (mg) of Norgestrel RS taken
M_{Sb}: Amount (mg) of Ethinylestradiol RS taken

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).
Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
Proceed as directed in the system suitability in the Assay.

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 Norgestrel and Ethinylestradiol Tablets is not less than 70%.

Start the test with 1 tablet of Norgestrel and Ethinylestradiol Tablets, withdraw not less than 50 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 exactly V mL of the subsequent filtrate, equivalent to about 17 µg of norgestrel (C$_{21}$H$_{28}$O$_2$) and about 1.7 µg of ethinylestradiol (C$_{20}$H$_{24}$O$_2$), transfer into a chromatography column [prepared by packing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 µm in particle diameter) in a tube about 1 cm in inside diameter]. After washing the column with 15 mL of water, elute with 3 mL of methanol, and evaporate the effluent in a water bath to dryness at about 40°C with the aid of a current air. Dissolve the residue in exactly 2 mL of diluted methanol (7 in 10), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Norgestrel RS and about 2.5 mg of Ethinylestradiol RS, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, then pipet 3 mL of this solution, add diluted methanol (7 in 10) 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. Determine the peak areas, A_{Ta} and A_{Tb}, of norgestrel and ethinylestradiol from the sample solution, and the peak areas, A_{Sa} and A_{Sb}, of norgestrel and ethinylestradiol from the standard solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of norgestrel (C}_{21}\text{H}_{28}\text{O}_2)$$
$$= M_{Sa} \times A_{Ta}/A_{Sa} \times 1/V \times 1/C_a \times 54$$

$$\text{Dissolution rate (\%) with respect to the labeled amount of ethinylestradiol (C}_{20}\text{H}_{24}\text{O}_2)$$
$$= M_{Sb} \times A_{Tb}/A_{Sb} \times 1/V \times 1/C_b \times 54$$

M_{Sa}: Amount (mg) of Norgestrel RS taken
M_{Sb}: Amount (mg) of Ethinylestradiol RS taken

C_a: Labeled amount (mg) of norgestrel (C$_{21}$H$_{28}$O$_2$) in 1 tablet
C_b: Labeled amount (mg) of ethinylestradiol (C$_{20}$H$_{24}$O$_2$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately not less than 20 Norgestrel and Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of norgestrel (C$_{21}$H$_{28}$O$_2$), add 4 mL of diluted methanol (7 in 10), add exactly 4 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size of not more than 0.2 µm, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of Norgestrel RS and about 5 mg of Ethinylestradiol RS, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 4 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. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

$$\text{Amount (mg) of norgestrel (C}_{21}\text{H}_{28}\text{O}_2)$$
$$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/50$$

$$\text{Amount (mg) of ethinylestradiol (C}_{20}\text{H}_{24}\text{O}_2)$$
$$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/50$$

M_{Sa}: Amount (mg) of Norgestrel RS taken
M_{Sb}: Amount (mg) of Ethinylestradiol RS taken

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).
Operating conditions—
Detector: Norgestrel—An ultraviolet absorption photometer (wavelength: 241 nm).
Ethinylestradiol—A fluorophotometer (excitation wavelength: 281 nm, fluorescence 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 (10 µm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of acetonitrile and water (11:9).
Flow rate: Adjust so that the retention time of norgestrel 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, ethinylestradiol, norgestrel and the internal standard are eluted in this order, and the resolution between the peaks of norgestrel and the internal standard is 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 ethinylestradiol and norgestrel to that of the internal standard are not more than 1.0%, respectively.

Containers and storage Containers—Tight containers.

Nortriptyline Hydrochloride

ノルトリプチリン塩酸塩
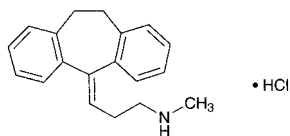
C$_{19}$H$_{21}$N.HCl: 299.84
3-(10,11-Dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methylpropylamine monohydrochloride
[*894-71-3*]

Nortriptyline Hydrochloride, when dried, contains not less than 98.5% of nortriptyline hydrochloride (C$_{19}$H$_{21}$N.HCl).

Description Nortriptyline Hydrochloride occurs as a white to yellowish white crystalline powder. It is odorless, or has a faint, characteristic odor.

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

The pH of a solution of 1.0 g of Nortriptyline Hydrochloride in 100 mL of water is about 5.5.

Melting point: 215 – 220°C

Identification **(1)** To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 mL of bromine TS: the color of the test solution disappears.

(2) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 to 2 drops of a solution of quinhydrone in methanol (1 in 40): a red color gradually develops.

(3) Determine the absorption spectrum of a solution of Nortriptyline 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.

(4) Determine the infrared absorption spectrum of Nortriptyline 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.

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

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Nortriptyline Hydrochloride in 10 mL of water: the solution is clear and colorless to very light yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Nortriptyline 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 Nortriptyline Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Nortriptyline Hydrochloride in 20 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform 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 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 cyclohexane, methanol and diethylamine (8:1:1) 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, 105°C, 2 hours).

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

Assay Weigh accurately about 0.5 g of Nortriptyline Hydrochloride, previously dried, dissolve in 5 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.
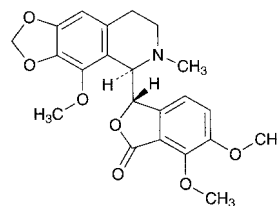
Each mL of 0.1 mol/L perchloric acid VS
= 29.98 mg of C$_{19}$H$_{21}$N.HCl

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Noscapine

Narcotine

ノスカピン

C$_{22}$H$_{23}$NO$_7$: 413.42
(3S)-6,7-Dimethoxy-3-[(5R)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-5-yl]isobenzofuran-1(3H)-one
[*128-62-1*]

Noscapine, when dried, contains not less than 98.5% of noscapine (C$_{22}$H$_{23}$NO$_7$).

Description Noscapine occurs as white, crystals or crystalline powder. It is odorless and tasteless.

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

Identification **(1)** Determine the absorption spectrum of a solution of Noscapine 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.

(2) Determine the infrared absorption spectrum of Noscapine, 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.

The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (See the General Notices 5.)

Optical rotation <2.49> $[\alpha]_D^{20}$: $+42 - +48°$ (after drying, 0.5 g, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 nm).

Melting point <2.60> 174 – 177°C

Purity (1) Chloride <1.03>—Dissolve 0.7 g of Noscapine in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 0.4 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 0.02%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Noscapine 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) Morphine—Dissolve 10 mg of Noscapine in 1 mL of water and 5 mL of 1-nitroso-2-naphthol TS with shaking, add 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the solution with 10 mL of chloroform, centrifuge, and collect the aqueous layer: the solution so obtained has no more color than a pale red.

(4) Related substances—Dissolve 0.7 g of Noscapine in 50 mL of acetone, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL. Pipet 5 mL of this 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 for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (60:60:9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute bismuth subnitrate-potassium iodide TS for spray 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% (2 g, 105°C, 4 hours).

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

Assay Weigh accurately about 0.8 g of Noscapine, previously dried, dissolve in 30 mL of acetic acid (100) and titrate <2.50> with 0.1 mol/L perchloric acid VS (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
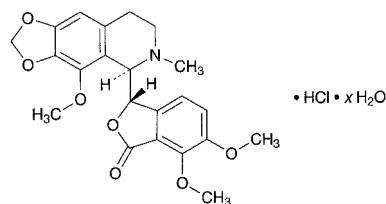= 41.34 mg of $C_{22}H_{23}NO_7$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Noscapine Hydrochloride Hydrate

Narcotine Hydrochloride

ノスカピン塩酸塩水和物

$C_{22}H_{23}NO_7.HCl.xH_2O$
(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one monohydrochloride hydrate
[*912-60-7*, anhydride]

Noscapine Hydrochloride Hydrate, when dried, contains not less than 98.0% of noscapine hydrochloride ($C_{22}H_{23}NO_7.HCl$: 449.88).

Description Noscapine Hydrochloride Hydrate occurs as colorless or white, crystals or crystalline powder. It is odorless, and has a bitter taste.

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

Identification (1) To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of formaldehyde-sulfuric acid TS: a purple color, changing to yellow-brown, is produced.

(2) To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of a solution of ammonium vanadate (V) in sulfuric acid (1 in 200): an orange color is produced.

(3) Dissolve 0.02 g of Noscapine Hydrochloride Hydrate in 1 mL of water, and add 3 drops of sodium acetate TS: a white, flocculent precipitate is produced.

(4) Dissolve 1 mg of Noscapine Hydrochloride Hydrate in 1 mL of diluted sulfuric acid (1 in 35), shake with 5 drops of a solution of disodium chromotropate dihydrate (1 in 50), and add 2 mL of sulfuric acid dropwise: a purple color is produced.

(5) Dissolve 0.1 g of Noscapine Hydrochloride Hydrate in 10 mL of water, make the solution alkaline with ammonia TS, and shake with 10 mL of chloroform. Separate the chloroform layer, wash with 5 mL of water, and filter. Distil most of the filtrate on a water bath, add 1 mL of ethanol (99.5), and evaporate to dryness. Dry the residue at 105°C for 4 hours: the residue so obtained melts <2.60> between 174°C and 177°C.

(6) Make a solution of Noscapine Hydrochloride Hydrate (1 in 50) alkaline with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity Morphine—Dissolve 10 mg of Noscapine Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

Loss on drying <2.41>   Not more than 9.0% (0.5 g, 120°C, 4 hours).

Residue on ignition <2.44>   Not more than 0.5% (1 g).

Assay   Weigh accurately about 0.5 g of Noscapine Hydrochloride Hydrate, 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
= 44.99 mg of $C_{22}H_{23}NO_7.HCl$

Containers and storage   Containers—Well-closed containers.
　　Storage—Light-resistant.

Nystatin

ナイスタチン

Nystatin is a mixture of polyene macrolide substances having antifungal activity produced by the growth of *Streptomyces noursei*.

It contains not less than 4600 units (potency) per mg, calculated on the dried basis. The potency of Nystatin is expressed as the unit of nystatin ($C_{47}H_{75}NO_{17}$: 926.09), and one unit corresponds to 0.27 μg of nystatin ($C_{47}H_{75}NO_{17}$).

Description   Nystatin occurs as a white to light yellow-brown powder.

It is soluble in formamide, sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification   (1)   Dissolve 1 mg of Nystatin in 5 mL of water and 1 mL of sodium hydroxide TS, heat for 2 minutes, and cool. To this solution add 3 mL of a solution of 4-aminoacetophenone in methanol (1 in 200) and 1 mL of hydrochloric acid: a red-purple color develops.

(2)   To 10 mg of Nystatin add 50.25 mL of a mixture of diluted methanol (4 in 5) and sodium hydroxide TS (200:1), heat at not exceeding 50°C to dissolve, then add diluted methanol (4 in 5) to make 500 mL. 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 Nystatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity   Heavy metals <1.07>—Proceed with 1.0 g of Nystatin 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).

Loss on drying <2.41>   Not more than 5.0% (0.3 g, in vacuum, 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—*Saccharomyces cerevisiae* ATCC 9763
　(ii)   Culture medium—Use the medium 2) under (1) Agar media for seed and base layer.

(iii)   Standard solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin RS equivalent to about 60,000 units, previously dried at 40°C for 2 hours in vacuum (not more than 0.67 kPa), dissolve in formamide to make a solution of 3000 units per 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 6.0) to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv)   Sample solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin equivalent to about 60,000 units, dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 300 units and 150 units, 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, and in a cold place.

Ofloxacin

オフロキサシン
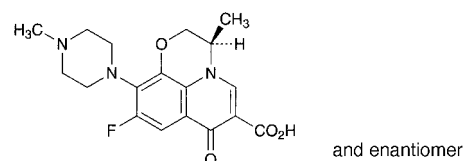
$C_{18}H_{20}FN_3O_4$: 361.37
(3RS)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid
[*82419-36-1*]

Ofloxacin, when dried, contains not less than 99.0% and not more than 101.0% of ofloxacin ($C_{18}H_{20}FN_3O_4$).

Description   Ofloxacin occurs as pale yellowish white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).

A soluton of Ofloxacin in sodium hydroxide TS (1 in 20) does not show optical rotation.

It is changed in color by light.

Melting point: about 265°C (with decomposition).

Identification   (1)   Determine the absorption spectrum of a solution of Ofloxacin in 0.1 mol/L hydrochloric acid TS (1 in 150,000) 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 Ofloxacin 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 Ofloxacin 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—Conduct this procedure without exposure to light. Dissolve 10 mg of Ofloxacin in 50 mL of a mixture of water and acetonitrile (6:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (6:1) 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: the area of the peak other than ofloxacin obtained from the sample solution is not larger than 2/5 times the peak area of ofloxacin obtained from the standard solution, and the total area of the peaks other than ofloxacin is not larger than the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 294 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 45°C.

Mobile phase: Dissolve 7.0 g of sodium perchlorate monohydrate and 4.0 g of ammonium acetate in 1300 mL of water, adjust the pH to 2.2 with phosphoric acid, and add 240 mL of acetonitrile.

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

Time span of measurement: About 1.8 times as long as the retention time of ofloxacin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Confirm that the peak area of ofloxacin 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: To 0.5 mL of the sample solution add 1 mL of a solution of ofloxacin demethyl substance in a mixture of water and acetonitrile (6:1) (1 in 20,000) and a mixture of water and acetonitrile (6:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ofloxacin is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.2% (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 Ofloxacin, 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.
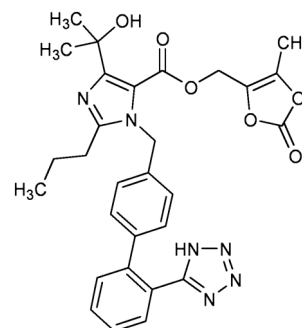
Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $C_{18}H_{20}FN_3O_4$

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

Olmesartan Medoxomil

オルメサルタン　メドキソミル

$C_{29}H_{30}N_6O_6$: 558.59
(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-imidazole-5-carboxylate
[*144689-63-4*]

Olmesartan Medoxomil contains not less than 98.5% and not more than 101.5% of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$), calculated on the anhydrous and residual solvent-free basis.

Description Olmesartan Medoxomil occurs as a white to pale yellowish white crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Olmesartan Medoxomil in acetonitrile (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 Olmesartan Medoxomil 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 Olmesartan Medoxomil 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 Olmesartan Medoxomil 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 Olmesartan Medoxomil 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 20 mg of Olmesartan Medoxomil 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, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention times of about 0.2 and about 1.6 to olmesartan medoxomil, obtained from the sample solution are not larger than 2/5 times and 3/10 times the peak area of olmesartan medoxomil obtained from the standard solution,

respectively, the area of the peaks other than olmesartan medoxomil and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of olmesartan medoxomil from the standard solution, and the total area of these peaks is not larger than 3/10 times the peak area of olmesartan medoxomil from the standard solution. In addition, the total area of the peaks other than olmesartan medoxomil from the sample solution is not larger than 4/5 times the peak area of olmesartan medoxomil from the standard solution. For the areas of the peaks, having the relative retention times of about 0.7 and about 3.4 to olmesartan medoxomil, multiply their relative response factors 0.65 and 1.39, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

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

Mobile phase A: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.5 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 400 mL of this solution add 100 mL of acetonitrile.

Mobile phase B: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.5 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 100 mL of this solution add 400 mL of 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	75	25
10 – 35	75 → 0	25 → 100
35 – 45	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: For 45 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add acetonitrile to make exactly 20 mL. Confirm that the peak area of olmesartan medoxomil 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 olmesartan medoxomil 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 olmesartan medoxomil is not more than 2.0%.

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 50 mg each of Olmesartan Medoxomil and Olmesartan Medoxomil RS (separately determine the water *<2.48>* and the residual solvent in the same

manners as Olmesartan Medoxomil), dissolve them separately in a mixture of acetonitrile and water (4:1) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add a mixture of water and acetonitrile (3:2) 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 calculate the ratios, Q_T and Q_S, of the peak area of olmesartan medoxomil to that of the internal standard.

Amount (mg) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$)
= $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Olmesartan Medoxomil RS taken, calculated on the anhydrous and residual solvent-free basis

*Internal standard solution—*A solution of isobutyl para-hydroxybenzoate in a mixture of water and acetonitrile (3:2) (1 in 2000).

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 40°C.

Mobile phase: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.4 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 330 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust so that the retention time of olmesartan medoxomil is about 16 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, olmesartan medoxomil 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 ratio of the peak area of olmesartan medoxomil to that of the internal standard is not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Olmesartan Medoxomil Tablets

オルメサルタン　メドキソミル錠

Olmesartan Medoxomil Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$: 558.59).

Method of preparation Prepare as directed under Tablets, with Olmesartan Medoxomil.

Identification To a quantity of powdered Olmesartan Medoxomil Tablets, equivalent to 20 mg of Olmesartan Medoxomil, add 60 mL of a mixture of acetonitrile and water (3:2), agitate for 10 minutes with the aid of ultrasonic

waves, and add a mixture of acetonitrile and water (3:2) to make 100 mL. Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (3:2) 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 255 nm and 259 nm.

Purity Related substances—To a quantity of powdered Olmesartan Medoxomil Tablets, equivalent to 20 mg of Olmesartan Medoxomil, add 20 mL of a mixture of acetonitrile and water (9:1), agitate for 15 minutes with the aid of ultrasonic waves, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (9:1) 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 the relative retention time of about 0.2 and about 1.6 to olmesartan medoxomil, obtained from the sample solution are not larger than 3/5 times the peak area of olmesartan medoxomil obtained from the standard solution, and the area of the peak other than olmesartan medoxomil and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of olmesartan medoxomil from the standard solution. Furthermore, the total area of the peaks other than olmesartan medoxomil from the sample solution is not larger than 1.4 times the peak area of olmesartan medoxomil from the standard solution. For the areas of the peaks, having the relative retention time of about 0.7 and about 3.4 to olmesartan medoxomil, multiply their relative response factors, 0.65 and 1.39, respectively.
Operating conditions—
Detector, column, column temperature, mobile phase, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Olmesartan Medoxomil.
Time span of measurement: For 45 minutes after injection, beginning after the solvent peak.
System suitability—
Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and water (9:1) to make exactly 20 mL. Confirm that the peak area of olmesartan medoxomil 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 olmesartan medoxomil are not less than 5500 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 olmesartan medoxomil 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 Olmesartan Medoxomil Tablets add $5V/7$ mL of a mixture of acetonitrile and water (3:2) and exactly $V/10$ mL of the internal standard solution. Agitate for 10 minutes with the aid of ultrasonic waves with occasional stir-

ring, and add a mixture of acetonitrile and water (3:2) to make V mL so that each mL contains about 0.2 mg of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$). Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (3:2) to make 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$)
   $= M_S \times Q_T/Q_S \times V/200$

M_S: Amount (mg) of Olmesartan Medoxomil RS taken, calculated on the anhydrous and residual solvent-free basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (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 2nd fluid for dissolution test as the dissolution medium, the dissolution rates in 30 minutes of 5-mg, 10-mg and 20-mg tablets are not less than 80%, and that in 30 minutes of 40-mg tablet is not less than 75%.
Start the test with 1 tablet of Olmesartan Medoxomil 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 6 μg of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Olmesartan Medoxomil RS (separately, determine the water <2.48> and the residual solvent in the same manner as Olmesartan Medoxomil), dissolve in 15 mL of ethanol (99.5) by warming at 50 – 60°C, and after cooling add ethanol (99.5) to make exactly 20 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL. Then, 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 257 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 olmesartan medoxomil ($C_{29}H_{30}N_6O_6$)
   $= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/4$

M_S: Amount (mg) of Olmesartan Medoxomil RS taken, calculated on the anhydrous and residual solvent-free basis
C: Labeled amount (mg) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Olmesartan Medoxomil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$), add 70 mL of a mixture of acetonitrile and water (3:2) and exactly 10 mL of the internal standard solution. Agitate for 15 minutes with the aid of ultrasonic waves with occasional stirring, and add a mixture of acetonitrile and water (3:2) to make 100 mL. Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (3:2) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Olmesartan Medoxomil RS (separately determine the water <2.48> and the residual sol-

vent in the same manner as Olmesartan Medoxomil), dissolve in 60 mL of a mixture of acetonitrile and water (3:2), add exactly 20 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 100 mL. To 5 mL of this solution add a mixture of acetonitrile and water (3:2) 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 olmesartan medoxomil to that of the internal standard.

Amount (mg) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$)
$$= M_S \times Q_T/Q_S \times 1/2$$

M_S: Amount (mg) of Olmesartan Medoxomil RS taken, calculated on the anhydrous and residual solvent-free basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (1 in 1000).
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 40°C.
Mobile phase: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.4 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 330 mL of this solution add 170 mL of acetonitrile.
Flow rate: Adjust so that the retention time of olmesartan medoxomil is about 16 minutes.
*System suitability*—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, olmesartan medoxomil 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 deviations of the ratio of the peak area of olmesartan medoxomil to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Olopatadine Hydrochloride

オロパタジン塩酸塩
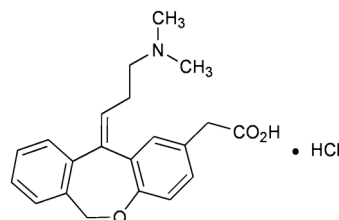
$C_{21}H_{23}NO_3$.HCl: 373.87
{11-[(1*Z*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepin-2-yl}acetic acid monohydrochloride
[*140462-76-6*]

Olopatadine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl).

Description   Olopatadine Hydrochloride occurs as white, crystals or crystalline powder.
It is very soluble in formic acid, sparingly soluble in water, and very slightly soluble in ethanol (99.5).
It dissolves in 0.01 mol/L hydrochloric acid TS.
The pH of a solution obtained by dissolving 1.0 g of Olopatadine Hydrochloride in 100 mL of water is 2.3 to 3.3.
Melting point: about 250°C (with decomposition).

Identification   **(1)**   Determine the absorption spectrum of a solution of Olopatadine Hydrochloride in 0.01 mol/L hydrochloric acid TS (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 Olopatadine 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)   To 5 mL of a solution of Olopatadine Hydrochloride (1 in 100) add 1 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity   **(1)**   Heavy metals <1.07>—Proceed with 2.0 g of Olopatadine 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 50 mg of Olopatadine Hydrochloride in 100 mL of a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) 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 olopatadine obtained from the sample solution is not larger than 1/10 times the peak area of olopatadine obtained from the standard solution.
Operating conditions—
Detector: An ultraviolet absorption photometer (wave-

length: 299 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 2.3 g of sodium lauryl sulfate in a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (11:9) to make 1000 mL.

Flow rate: Adjust so that the retention time of olopatadine is about 11 minutes.

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) to make exactly 20 mL. Confirm that the peak area of olopatadine 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: 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 olopatadine 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 olopatadine is not more than 2.0%.

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 0.5 g of Olopatadine Hydrochloride, previously dried, dissolve in 3 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
= 37.39 mg of $C_{21}H_{23}NO_3.HCl$

Containers and storage Containers—Well-closed containers.

Olopatadine Hydrochloride Tablets

オロパタジン塩酸塩錠

Olopatadine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of olopatadine hydrochloride ($C_{21}H_{23}NO_3.HCl$: 373.87).

Method of preparation Prepare as directed under Tablets, with Olopatadine Hydrochloride.

Identification Shake well a quantity of powdered Olopatadine Hydrochloride Tablets, equivalent to 5 mg of Olopatadine Hydrochloride, with 100 mL of 0.01 mol/L hydrochloric acid TS, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry $<2.24>$: it exhibits a maximum between

295 nm and 299 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 Olopatadine Hydrochloride Tablets add $4V/5$ mL of a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2). To this solution add exactly $V/10$ mL of the internal standard solution, shake well, and add a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) to make V mL so that each mL contains about 50 μg of olopatadine hydrochloride ($C_{21}H_{23}NO_3.HCl$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use this filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of olopatadine hydrochloride
($C_{21}H_{23}NO_3.HCl$)
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of olopatadine hydrochloride for assay taken

Internal standard solution—A solution of doxepin hydrochloride in a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) (7 in 20,000).

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 15 minutes of Olopatadine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Olopatadine Hydrochloride Tablets, 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, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of olopatadine hydrochloride ($C_{21}H_{23}NO_3.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of olopatadine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL. Then pipet 10 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 olopatadine in each solution.

Dissolution rate (%) with respect to the labeled amount of olopatadine hydrochloride ($C_{21}H_{23}NO_3.HCl$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of olopatadine hydrochloride for assay taken
C: Labeled amount (mg) of olopatadine hydrochloride ($C_{21}H_{23}NO_3.HCl$) 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 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of olopatadine are not less than 10,000 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 olopatadine is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Olopatadine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl), add 80 mL of a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2), and add exactly 10 mL of the internal standard solution. Shake well for 10 minutes, add a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) to make 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of olopatadine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) 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 olopatadine to that of the internal standard.

$$\text{Amount (mg) of olopatadine hydrochloride}$$
$$(C_{21}H_{23}NO_3.HCl)$$
$$= M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of olopatadine hydrochloride for assay taken

Internal standard solution—A solution of doxepin hydrochloride in a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (3:2) (7 in 20,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 299 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 2.3 g of sodium lauryl sulfate in a mixture of 0.05 mol/L phosphate buffer solution (pH 3.5) and acetonitrile (11:9) to make 1000 mL.
Flow rate: Adjust so that the retention time of olopatadine 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, olopatadine and the internal standard 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of olopatadine is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Omeprazole

オメプラゾール

and enantiomer
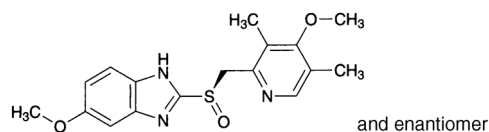
$C_{17}H_{19}N_3O_3S$: 345.42
(RS)-5-Methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl}-1H-benzimidazole
[73590-58-6]

Omeprazole, when dried, contains not less than 99.0% and not more than 101.0% of omeprazole ($C_{17}H_{19}N_3O_3S$).

Description Omeprazole occurs as a white to yellowish white crystalline powder.
It is freely soluble in N,N-dimethylformamide, sparingly soluble in ethanol (99.5), and practically insoluble in water.
A solution of Omeprazole in N,N-dimethylformamide (1 in 25) shows no optical rotation.
It gradually turns yellowish white on exposure to light.
Melting point: about 150°C (with decomposition).

Identification (1) Add phosphate buffer solution (pH 7.4) to 1 mL of a solution of Omeprazole in ethanol (99.5) (1 in 1000) 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 Omeprazole 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 Omeprazole in 25 mL of N,N-dimethylformamide: the solution is clear and colorless or light yellow. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: the absorbance at 420 nm is not more than 0.3.
(2) Heavy metals *<1.07>*—Proceed with 2.0 g of Omeprazole 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 the procedure soon after preparation of the sample solution. Dissolve 50 mg of Omeprazole in 50 mL of the mobile phase, 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 of the peak areas of the sample solution by the automatic integration method, and calculate the amounts of them by the area percentage method: each of the amount of the peaks other than omeprazole is not more than 0.1%, and the total amount of the peaks other than omeprazole is not more than 0.5%.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diame-

ter 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: Dissolve 2.83 g of disodium hydrogen phosphate dodecahydrate and 0.21 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL. If necessary, adjust the pH to 7.6 with diluted phosphoric acid (1 in 100). Add 11 volumes of acetonitrile to 29 volumes of this solution.

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

Time span of measurement: About 10 times as long as the retention time of omeprazole, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 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 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 the mobile phase to make exactly 25 mL. Confirm that the peak area of omeprazole obtained from 10 μL of this solution is equivalent to 15 to 25% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Omeprazole and 25 mg of 1,2-dinitrobenzene in 5 mL of sodium borate solution (19 in 5000) and 95 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above conditions, omeprazole and 1,2-dinitrobenzene 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of omeprazole is not more than 2.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 2 hours).

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

Assay Weigh accurately about 0.4 g of Omeprazole, previously dried, dissolve in 70 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination using the same method on a solution consisting of 70 mL of *N,N*-dimethylformamide and 12 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 34.54 mg of $C_{17}H_{19}N_3O_3S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, in a cold place.

Omeprazole Delayed-release Tablets

オメプラゾール腸溶錠

Omeprazole Delayed-release Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$: 345.42).

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

Identification Powder Omeprazole Delayed-release

Tablets. To a portion of the powder, equivalent to 10 mg of Omeprazole, add 10 mL of ethanol (95), shake for 10 minutes, and centrifuge. To 1 mL of the supernatant liquid add phosphate buffer solution (pH 7.4) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 273 nm and 277 nm, and between 299 nm and 303 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 Omeprazole Delayed-release Tablets add $V/20$ mL of a solution of sodium tetraborate decahydrate (19 in 5000), and shake thoroughly to disintegrate the tablet. Then, proceed as directed in the Assay.

Amount (mg) of omeprazole ($C_{17}H_{19}N_3O_3S$)
$$= M_S \times Q_T/Q_S \times V/50$$

M_S: Amount (mg) of omeprazole for assay taken

Internal standard solution—A solution of 1,2-dinitrobenzene in ethanol (95) (1 in 400).

Dissolution <6.10> When the tests are performed at 50 revolutions per minute according to the Paddle method, using 900 mL each of 1st fluid for dissolution test and 2nd fluid for dissolution test as the dissolution medium, the dissolution rates of 10-mg tablet and 20-mg tablet in 120 minutes of the test using the 1st fluid for dissolution test are not more than 5%, respectively, and those of 10-mg tablet in 20 minutes and 20-mg tablet in 15 minutes of the test using the 2nd fluid for dissolution test are not less than 85%, respectively.

Start the test with 1 tablet of Omeprazole Delayed-release 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 11 μg of omeprazole ($C_{17}H_{19}N_3O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of omeprazole for assay, previously dried in vacuum at 50°C using phosphorus (V) oxide as desiccant for 2 hours, and dissolve in ethanol (95) 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 as directed under Ultraviolet-visible Spectrophotometry <2.24> at 323 nm when the test is performed using the 1st fluid as the dissolution medium and at 293 nm when the test is performed using the 2nd fluid as the dissolution medium, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of omeprazole for assay taken
C: Labeled amount (mg) of omeprazole ($C_{17}H_{19}N_3O_3S$) in 1 tablet

Assay To 20 Omeprazole Delayed-release Tablets add $V/20$ mL of a solution of sodium tetraborate decahydrate (19 in 5000), shake to disintegrate. To this solution add $3V/5$ mL of ethanol (95), shake for 15 minutes, then add ethanol (95) to make exactly V mL so that each mL contains about 0.4 mg of omeprazole ($C_{17}H_{19}N_3O_3S$), and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 4 mL of the inter-

nal standard solution, add a mixture of ethanol (95) and a solution of sodium tetraborate decahydrate (19 in 5000) (19:1) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of omeprazole for assay, previously dried in vacuum at 50°C with phosphorus (V) oxide as the desiccant for 2 hours, dissolve in a mixture of ethanol (95) and a solution of sodium tetraborate decahydrate (19 in 5000) (19:1), add exactly 20 mL of the internal standard solution, add a mixture of ethanol (95) and a solution of sodium tetraborate decahydrate (19 in 5000) (19:1) to make 100 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 omeprazole to that of the internal standard.

Amount (mg) of omeprazole ($C_{17}H_{19}N_3O_3S$) in tablet
$$= M_S \times Q_T/Q_S \times V/1000$$

M_S: Amount (mg) of omeprazole for assay taken

Internal standard solution—A solution of 1,2-dinitrobenzene in ethanol (95) (1 in 400).
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 octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2.83 g of disodium hydrogen phosphate dodecahydrate and 0.21 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 7.6 with diluted phosphoric acid (1 in 100). To 290 mL of this solution add 110 mL of acetonitrile.
Flow rate: Adjust so that the retention time of omeprazole is about 8 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, omeprazole 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 omeprazole to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Opium Alkaloids Hydrochlorides

アヘンアルカロイド塩酸塩

Opium Alkaloids Hydrochlorides consist of the hydrochlorides of some of the main alkaloids obtained from opium.
It contains not less than 47.0% and not more than 52.0% of morphine ($C_{17}H_{19}NO_3$: 285.34), and not less than 35.0% and not more than 41.0% of other opium alkaloids.

Description   Opium Alkaloids Hydrochlorides occur as a white to light brown powder.
It is soluble in water, and slightly soluble in ethanol (99.5).

It is colored by light.

Identification   (1)   Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Separately, dissolve 60 mg of Morphine Hydrochloride Hydrate, 40 mg of Noscapine Hydrochloride Hydrate, 10 mg of Codein Phosphate Hydrate and 10 mg of Papaverine Hydrochloride in 10 mL each of diluted ethanol (1 in 2), and use these solutions as the standard solutions (1), (2), (3) and (4), 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 solutions 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 solution (28) (20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): each spot from the sample solution is the same in color tone and Rf value with the corresponding spot from the standard solutions (1), (2), (3) and (4) (morphine, noscapine, codeine and papaverine).

(2)   A solution of Opium Alkaloids Hydrochlorides (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54>   Dissolve 1.0 g of Opium Alkaloids Hydrochlorides in 50 mL of water: the pH of the solution is between 3.0 and 4.0.

Purity   (1)   Clarity and color of solution—Dissolve 0.5 g of Opium Alkaloids Hydrochlorides in 10 mL of water: the solution is clear, and its absorbance <2.24> at 420 nm is not more than 0.20.

(2)   Meconic acid—Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 2 mL of water, and pour into a polyethylene column 1 cm in inside diameter, packed with about 0.36 g of aminopropylsilanized silica gel for pretreatment (55 – 105 μm in particle diameter) and previously washed through with 5 mL of water. Then, wash the column with 5 mL of water, 5 mL of methanol and 10 mL of 0.1 mol/L hydrochloric acid in this order, then elute with 2 mL of 1 mol/L hydrochloric acid, and use the eluate as the test solution. To the test solution add 2 mL of dilute sodium hydroxide TS and 1 drop of iron (III) chloride TS: no red color develops.

Loss on drying <2.41>   Not more than 6.0% (0.5 g, 120°C, 8 hours).

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

Assay   Weigh accurately about 0.1 g of Opium Alkaloids Hydrochlorides, and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of morphine hydrochloride hydrate for assay, dissolve in 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 the peak areas of morphine, codeine, papaverine, thebaine, narceine and noscapine, A_{T1}, A_{T2}, A_{T3}, A_{T4}, A_{T5} and A_{T6}, from the sample solution, and the peak area of morphine, A_S, from the standard solution.

Amount (mg) of morphine ($C_{17}H_{19}NO_3$)
$$= M_S \times A_{T1}/A_S \times 0.887$$

Amount (mg) of other opium alkaloids
$$= M_S \times \{(A_{T2} + 0.29A_{T3} + 0.20A_{T4} + 0.19A_{T5} + A_{T6})/A_S\} \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

The relative retention time of codine, papaverine, thebaine, narceine and noscapine to morphine obtained under the following operating conditions are as follows.

Component	Relative retention time
codeine	1.1
papaverine	1.9
thebaine	2.5
narceine	2.8
noscapine	3.6

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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, and mix.

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

System suitability—

System performance: Dissolve 60 mg of Morphine Hydrochloride Hydrate, 10 mg of Codeine Phosphate Hydrate, 10 mg of Papaverine Hydrochloride and 40 mg of Noscapine Hydrochloride Hydrate in water to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, morphine, codeine, papaverine and noscapine are eluted in this order with the complete separation between these peaks and with the resolution between the peaks of morphine and codeine 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 morphine is not more than 1.0%.

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

Opium Alkaloids Hydrochlorides Injection

アヘンアルカロイド塩酸塩注射液

Opium Alkaloids Hydrochlorides Injection is an aqueous injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description   Opium Alkaloids Hydrochlorides Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification   To 1 mL of Opium Alkaloids Hydrochlorides Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution, and proceed as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

Extractable volume <6.05>   It meets the requirement.

Assay   Pipet 2 mL of Opium Alkaloids Hydrochlorides Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride hydrate for assay, and dissolve in exactly 10 mL of the internal standard solution, add water 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 morphine to that of the internal standard.

$$\text{Amount (mg) of morphine } (C_{17}H_{19}NO_3) = M_S \times Q_T/Q_S \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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, and mix.

Flow rate: Adjust so that the retention time of morphine 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, morphine 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 ratios of the peak area of morphine 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.

Opium Alkaloids and Atropine Injection

アヘンアルカロイド・アトロピン注射液

Opium Alkaloids and Atropine Injection is an aqueous injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2$. $H_2SO_4.H_2O$: 694.84].

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description   Opium Alkaloids and Atropine Injection is a colorless or light brown, clear liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification   (1)   To 1 mL of Opium Alkaloids and Atropine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2)   To 2 mL of Opium Alkaloids and Atropine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Atropine Sulfate RS in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained 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 and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.2 *R*f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same *R*f value (atropine).

Extractable volume <6.05>   It meets the requirements.

Assay   (1)   Morphine—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride hydrate for assay, dissolve in 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 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 morphine to that of the internal standard.

Amount (mg) of morphine ($C_{17}H_{19}NO_3$)
$$= M_S \times Q_T/Q_S \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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, and mix.

Flow rate: Adjust so that the retention time of morphine 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, morphine 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 ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2)   Atropine sulfate hydrate—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 2 mL of the internal standard solution, and add 10 mL of diluted dilute hydrochloric acid (1 in 10). Shake this solution with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (determine separately the loss on drying <2.41> under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, 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 Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of atropine to that of the internal standard.

Amount (mg) of atropine sulfate hydrate
[$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$]
$$= M_S \times Q_T/Q_S \times 1/50 \times 1.027$$

M_S: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

Internal standard solution—A solution of homatropine

hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of atropine is about 5 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 atropine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 2.0%.

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

Storage—Light-resistant.

Opium Alkaloids and Scopolamine Injection

アヘンアルカロイド・スコポラミン注射液

Opium Alkaloids and Scopolamine Injection is an aqueous injection.

It contains not less than 1.80 w/v% and not more than 2.20 w/v% of morphine (C$_{17}$H$_{19}$NO$_3$: 285.34) and not less than 0.054 w/v% and not more than 0.066 w/v% of scopolamine hydrobromide hydrate (C$_{17}$H$_{21}$NO$_4$.HBr.3H$_2$O: 438.31).

Method of preparation

Opium Alkaloids Hydrochlorides	40 g
Scopolamine Hydrobromide Hydrate	0.6 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description　Opium Alkaloids and Scopolamine Injection is a clear, colorless to light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification　**(1)**　To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2)　To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow

to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 mL of water. To 2 mL of this solution add 2 mL of ammonia TS, proceed with this solution in the same manner as for the sample solution, and use a solution so obtained 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 and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 *R*f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same *R*f value (scopolamine).

Extractable volume <6.05>　It meets the requirements.

Assay　**(1)**　Morphine—Pipet 1 mL of Opium Alkaloids and Scopolamine Injection, add 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride hydrate for assay, dissolve in exactly 10 mL of the internal standard solution, add water 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 morphine to that of the internal standard.

$$\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_3)$$
$$= M_S \times Q_T/Q_S \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of etilefrin hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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, and mix.

Flow rate: Adjust so that the retention time of morphine 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, morphine 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 ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2)　Scopolamine hydrobromide hydrate—Pipet 2 mL of Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution

add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scoporamine Hydrobromide RS (determine separately the loss on drying <2.41> under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use thus obtained solution as the standard solution. 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 scopolamine to that of the internal standard.

Amount (mg) of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$)
= $M_S \times Q_T/Q_S \times 1/50 \times 1.141$

M_S: Amount (mg) of Scopolamine Hydrobromide RS taken, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.
Column temperature: A constant temperature of about 210°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust so that the retention time of scopolamine 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, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

Containers and storage   Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Weak Opium Alkaloids and Scopolamine Injection

弱アヘンアルカロイド・スコポラミン注射液

Weak Opium Alkaloids and Scopolamine Injection is an aqueous injection.
It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34) and not less than 0.027 w/v% and not more than 0.033 w/v% of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$: 438.31).

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Scopolamine Hydrobromide Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description   Weak Opium Alkaloids and Scopolamine Injection is a clear, colorless or light brown liquid.
It is affected by light.
pH: 2.5 – 3.5

Identification   (1)   To 1 mL of Weak Opium Alkaloids and Scopolamine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2)   To 2 mL of Weak Opium Alkaloids and Scopolamine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide RS in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained 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 and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 Rf value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same Rf value (scopolamine).

Extractable volume <6.05>   It meets the requirements.

Assay   (1)   Morphine—Pipet 2 mL of Weak Opium Alkaloids and Scopolamine Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride hydrate for assay, dissolve in exactly 10 mL of the internal standard solution, add water 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 morphine to that of the internal standard.

$$\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_3)$$
$$= M_S \times Q_T \times Q_S \times 0.887$$

M_S: Amount (mg) of morphine hydrochloride hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrin hydrochloride (1 in 500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 285 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, and mix.
Flow rate: Adjust so that the retention time of morphine 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, morphine 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 ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 4 mL of Weak Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scoporamine Hydrobromide RS (separately determine the loss on drying <2.41> under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use so obtained solution as the standard solution. 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 scopolamine to that of the internal standard.

$$\text{Amount (mg) of scopolamine hydrobromide hydrate}$$
$$(\text{C}_{17}\text{H}_{21}\text{NO}_4.\text{HBr}.3\text{H}_2\text{O})$$
$$= M_S \times Q_T/Q_S \times 1/50 \times 1.141$$

M_S: Amount (mg) of Scopolamine Hydrobromide RS taken, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenylmethyl silicone polymer for gas chromatography.
Column temperature: A constant temperature of about 210°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust so that the retention time of scopolamine 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, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Orciprenaline Sulfate

オルシプレナリン硫酸塩
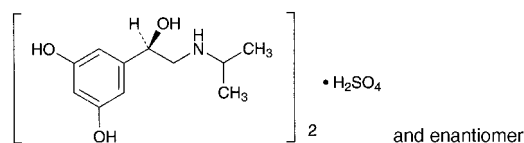
$(\text{C}_{11}\text{H}_{17}\text{NO}_3)_2.\text{H}_2\text{SO}_4$: 520.59
5-{(1*RS*)-1-Hydroxy-
2-[(1-methylethyl)amino]ethyl}benzene-1,3-diol
hemisulfate
[*5874-97-5*]

Orciprenaline Sulfate contains not less than 98.5% of orciprenaline sulfate [$(\text{C}_{11}\text{H}_{17}\text{NO}_3)_2.\text{H}_2\text{SO}_4$], calculated on the dried basis.

Description Orciprenaline Sulfate occurs as white, crystals or crystalline powder.
It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.
A solution of Orciprenaline Sulfate (1 in 20) shows no optical rotation.
Melting point: about 220°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS (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 Orci-

prenaline Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1607 cm^{-1}, 1153 cm^{-1}, 1131 cm^{-1} and 1110 cm^{-1}.

(3) A solution of Orciprenaline Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Orciprenaline Sulfate in 10 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 Orciprenaline Sulfate in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3 mL of Matching Fluid T add 1 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Orciprenaline Sulfate 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) Orciprenalone—Dissolve 0.200 g of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 328 nm is not more than 0.075.

Loss on drying <2.41> Not more than 1.5% (1 g, in vacuum, 105°C, 4 hours).

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

Assay Weigh accurately about 0.7 g of Orciprenaline Sulfate, dissolve in 100 mL of acetic acid (100) by warming on a water bath, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 52.06 mg of $(C_{11}H_{17}NO_3)_2.H_2SO_4$

</div>

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

Oxapium Iodide

オキサピウムヨウ化物
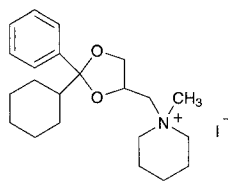
C$_{22}$H$_{34}$INO$_2$: 471.42
1-(2-Cyclohexyl-2-phenyl-1,3-dioxolan-4-ylmethyl)-1-methylpiperidinium iodide
[*6577-41-9*]

Oxapium Iodide, when dried, contains not less than 98.5% of oxapium iodide (C$_{22}$H$_{34}$INO$_2$).

Description Oxapium Iodide occurs as a white crystalline powder.

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

A solution of Oxapium Iodide in methanol (1 in 100) does not show optical rotation.

Identification (1) Determine the infrared absorption spectrum of Oxapium Iodide, previously dried, 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.

(2) Dissolve 0.1 g of Oxapium Iodide in 10 mL of methanol, and add 2 mL of dilute nitric acid and 2 mL of silver nitrate TS: a greenish yellow precipitate is formed.

Melting point <2.60> 198 – 203°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Oxapium Iodide 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 0.05 g of Oxapium Iodide in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) 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. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than oxapium from the sample solution is not larger than the area of the peak of oxapium from the standard solution.

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 20°C to 30°C.

Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine add water to make 1000 mL. To 50 mL of this solution add 500 mL of acetonitril, 10 mL of dilute acetic acid and 440 mL of water.

Flow rate: Adjust so that the retention time of oxapium is about 4 minutes.

Selection of column: Dissolve 0.05 g of Oxapium Iodide and 3 mg of benzophenone in 100 mL of the mobile phase. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of oxapium and benzophenone in this order with the resolution between these peaks being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of oxapium obtained from 50 μL of the standard solution composes 5 to 15% of the full scale.

Time span of measurement: About 6 times as long as the retention time of oxapium, beginning after the peak of iodide ion.

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 Oxapium Iodide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (9:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration, platinum electrode). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.14 mg of $C_{22}H_{34}INO_2$

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

Oxaprozin

オキサプロジン
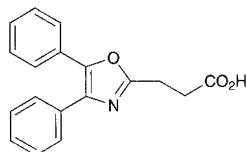
$C_{18}H_{15}NO_3$: 293.32
3-(4,5-Diphenyloxazol-2-yl)propanoic acid
[*21256-18-8*]

Oxaprozin, when dried, contains not less than 98.5% of oxaprozin ($C_{18}H_{15}NO_3$).

Description　Oxaprozin occurs as a white to yellowish white crystalline powder.

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

It is gradually affected by light.

Identification　Determine the infrared absorption spectrum of Oxaprozin, 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>　$E_{1\,cm}^{1\%}$ (285 nm): 455 – 495 (after drying, 10 mg, methanol, 1000 mL).

Melting point <2.60>　161 – 165°C

Purity　**(1)**　Heavy metals <1.07>—Proceed with 2.0 g of Oxaprozin 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 Oxaprozin according to Method 3, and perform the test (not more than 1 ppm).

(3)　Related substances—Dissolve 0.10 g of Oxaprozin 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 (1). Pipet 5 mL, 3 mL and 1 mL of the standard solution (1), add methanol to each to make exactly 10 mL, and use these solutions as the standard solutions (2), (3) and (4), 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 solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the total intensity of the spots other than the principal spot from the sample solution is not more than 1.0% calculated on the basis of intensities of the spots from the standard solutions (1), (2), (3) and (4).

Loss on drying <2.41>　Not more than 0.3% (1 g, 105°C,

2 hours).

**Residue on ignition** <2.44>　Not more than 0.3% (1 g).

Assay　Weigh accurately about 0.5 g of Oxaprozin, previously dried, dissolve in 50 mL of ethanol (95), 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
= 29.33 mg of $C_{18}H_{15}NO_3$

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

Oxazolam

オキサゾラム
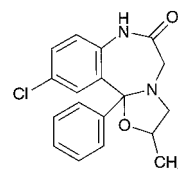
$C_{18}H_{17}ClN_2O_2$: 328.79
10-Chloro-2-methyl-11b-phenyl-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one
[*24143-17-7*]

Oxazolam, when dried, contains not less than 99.0% of oxazolam ($C_{18}H_{17}ClN_2O_2$).

Description　Oxazolam occurs as white, crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), soluble in 1,4-dioxane and in dichloromethane, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It gradually changes in color by light.

Melting point: about 187°C (with decomposition).

Identification　**(1)**　Dissolve 0.01 g of Oxazolam in 10 mL of ethanol (95) by heating, and add 1 drop of hydrochloric acid: a light yellow color develops, and the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

(2)　Dissolve 0.01 g of Oxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3)　Place 2 g of Oxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of 6 mol/L hydrochloric acid TS, and boil under a reflux condenser for 5 hours. After cooling, neutralize with a solution of sodium hydroxide (1 in 4), and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 20 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry in vacuum at 60°C for 1 hour: the crystals melt <2.60> between 96°C and 100°C.

(4)　Determine the absorption spectrum of a solution of Oxazolam 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.

(5)   Proceed with Oxazolam as directed under Flame Coloration Test <1.04> (2), and perform the test: a green color appears.

Absorbance <2.24> $E_{1\,cm}^{1\%}$ (246 nm): 410 – 430 (after drying, 1 mg, ethanol (95), 100 mL).

Purity   (1)   Chloride <1.03>—To 1.0 g of Oxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this 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 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Oxazolam 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>—Place 1.0 g of Oxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate monohydrate solution, heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(4)   Related substances—Dissolve 0.05 g of Oxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane 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. Immediately air-dry, develop the plate with a mixture of toluene and acetone (8:1) 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% (1 g, 105°C, 3 hours).

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

Assay   Weigh accurately about 0.65 g of Oxazolam, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and 1,4-dioxane (1:1). 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, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.88 mg of $C_{18}H_{17}ClN_2O_2$

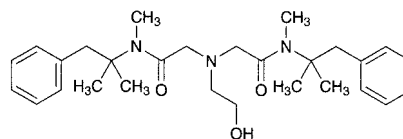**Containers and storage**   Containers—Tight containers.
Storage—Light-resistant.

Oxethazaine

Oxetacaine

オキセサゼイン

$C_{28}H_{41}N_3O_3$: 467.64
2,2′-(2-Hydroxyethylimino)bis[*N*-(1,1-dimethyl-2-phenylethyl)-*N*-methylacetamide]
[*126-27-2*]

Oxethazaine, when dried, contains not less than 98.5% of oxethazaine ($C_{28}H_{41}N_3O_3$).

Description   Oxethazaine occurs as a white to pale yellowish white crystalline powder.

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

Identification   (1)   Determine the absorption spectrum of a solution of Oxethazaine in ethanol (95) (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 Oxethazaine 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.

Melting point <2.60>   101 – 104°C

Purity   (1)   Chloride <1.03>—Dissolve 1.0 g of Oxethazaine in 20 mL of ethanol (95), 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, 20 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

(2)   Heavy metals <1.07>—Proceed with 2.0 g of Oxethazaine 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 0.40 g of Oxethazaine in 10 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 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 isopropylether, tetrahydrofuran, methanol and ammonia solution (28) (24:10:5:1) 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.

(4)   2-Aminoethanol—To 1.0 g of Oxethazaine add

methanol to make exactly 10 mL, then add 0.1 mL of a solution of 1-fluoro-2,4-dinitrobenzene in methanol (1 in 25), shake well, and heat at 60°C for 20 minutes: the solution has no more color than the following control solution.

Control solution: To 0.10 g of 2-aminoethanol add methanol to make exactly 200 mL, pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Proceed as directed above.

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 0.9 g of Oxethazaine, 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.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 46.76 \text{ mg of } C_{28}H_{41}N_3O_3$$

Containers and storage Containers—Tight containers.

Oxprenolol Hydrochloride

オクスプレノロール塩酸塩
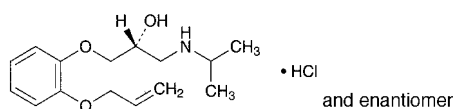
$C_{15}H_{23}NO_3.HCl: 301.81$
(2*RS*)-1-[2-(Allyloxy)phenoxy]-
3-(1-methylethyl)aminopropan-2-ol monohydrochloride
[*6452-73-9*]

Oxprenolol Hydrochloride, when dried, contains not less than 98.5% of oxprenolol hydrochloride ($C_{15}H_{23}NO_3.HCl$).

Description Oxprenolol Hydrochloride occurs as a white crystalline powder.

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

Identification (1) To 2 mL of a solution of Oxprenolol Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer, and a blue-purple color develops in the water layer.

(2) To 3 mL of a solution of Oxprenolol Hydrochloride (1 in 150) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Oxprenolol 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 Oxprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

Melting point <2.60> 107 – 110°C

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

(2) Heavy metals <1.07>—Proceed with 2.0 g of Oxprenolol 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).

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

(4) Related substances—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL of the sample 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 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 in a developing chamber saturated with ammonia vapor with a mixture of chloroform and methanol (9:1) 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% (1 g, 80°C, 3 hours).

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

Assay Weigh accurately about 0.6 g of Oxprenolol 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.
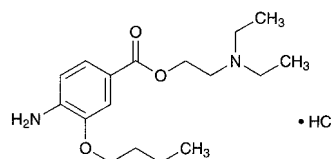
$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 30.18 \text{ mg of } C_{15}H_{23}NO_3.HCl$$

Containers and storage Containers—Tight containers.

Oxybuprocaine Hydrochloride

Benoxinate Hydrochloride

オキシブプロカイン塩酸塩

$C_{17}H_{28}N_2O_3.HCl: 344.88$
2-(Diethylamino)ethyl 4-amino-3-butyloxybenzoate monohydrochloride
[*5987-82-6*]

Oxybuprocaine Hydrochloride, when dried, contains not less than 99.0% of oxybuprocaine hydrochloride ($C_{17}H_{28}N_2O_3.HCl$).

Description Oxybuprocaine Hydrochloride occurs as

white, crystals or crystalline powder. It is odorless, and has a saline taste. It exhibits anesthetic properties when placed on the tongue.

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

The pH of a solution of 1.0 g of Oxybuprocaine Hydrochloride in 10 mL of water is between 5.0 and 6.0.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Oxybuprocaine Hydrochloride 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 Oxybuprocaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: an oily substance is produced. Rub the inner surface of the container with a glass rod: white crystals are formed. Collect the crystals so obtained, recrystallize from water, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 5 hours: the crystals melt <*2.60*> between 103°C and 106°C.

(3) Determine the absorption spectrum of a solution of Oxybuprocaine 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.

(4) A solution of Oxybuprocaine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 158 – 162°C

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

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Oxybuprocaine 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) Related substances—Dissolve 0.25 g of Oxybuprocaine Hydrochloride in 10 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 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, ethanol (95) and formic acid (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde 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% (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 Oxybuprocaine 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.
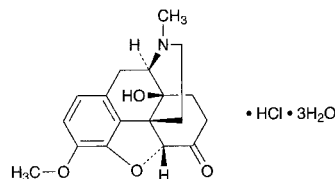
Each mL of 0.1 mol/L perchloric acid VS
= 34.49 mg of $C_{17}H_{28}N_2O_3.HCl$

Containers and storage Containers—Well-closed contain-

ers.

Storage—Light-resistant.

Oxycodone Hydrochloride Hydrate

オキシコドン塩酸塩水和物

$C_{18}H_{21}NO_4.HCl.3H_2O$: 405.87
(5*R*)-4,5-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one monohydrochloride trihydrate
[*124-90-3*, anhydride]

Oxycodone Hydrochloride Hydrate contains not less than 98.0% and not more than 101.0% of oxycodone hydrochloride ($C_{18}H_{21}NO_4.HCl$: 351.83), calculated on the anhydrous basis.

Description Oxycodone Hydrochloride Hydrate occurs as a white crystalline powder.

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

The pH of a solution dissolved 1.0 g of Oxycodone Hydrochloride Hydrate in 10 mL of water is between 3.8 and 5.8.

It is affected by light.

Identification **(1)** Determine the absorption spectrum of a solution of Oxycodone 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 Oxycodone 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 Oxycodone Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: $-140 – -149°$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 26 mg of Oxycodone Hydrochloride Hydrate in 20 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A 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. Determine each peak area by the automatic integration method: the area of the peak other than oxycodone obtained from the sample solution is not larger than 1/5 times the peak area of oxycodone obtained from the standard solution, and the total area

of the peaks other than oxycodone from the sample solution is not larger than 3/5 times the peak area of oxycodone from the standard solution. For the area of the peak, having the relative retention time of about 1.8 to oxycodone, multiply the relative response factor 0.17.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 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: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust to pH 3.0 with sodium hydroxide TS. To 4 volumes of this solution add 1 volume of tetrahydrofuran for liquid chromatography.

Mobile phase B: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust to pH 3.0 with sodium hydroxide TS. To 1 volume of this solution add 1 volume of tetrahydrofuran 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 – 30	100	0
30 – 70	100 → 0	0 → 100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 5 times as long as the retention time of oxycodone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2.5 mL of the standard solution, add the mobile phase A to make exactly 50 mL. Confirm that the peak area of oxycodone obtained with 50 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 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 oxycodone are not less than 3000 and between 0.7 and 1.3, 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 oxycodone is not more than 2.0%.

Water *<2.48>* 12 – 15% (0.2 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 Oxycodone 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
= 35.18 mg of $C_{18}H_{21}NO_4 \cdot HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Compound Oxycodone Injection

Compound Hycodenone Injection

複方オキシコドン注射液

Compound Oxycodone Injection is an aqueous injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$: 405.87), and not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O$: 275.73).

Method of preparation

Oxycodone Hydrochloride Hydrate	8 g
Hydrocotarnine Hydrochloride Hydrate	2 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Compound Oxycodone Injection is a clear, colorless to pale yellow liquid.

It is affected by light.

pH: 2.5 – 4.0

Identification (1) To 1 mL of Compound Oxycodone Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

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

Assay Pipet 2 mL of Compound Oxycodone Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride hydrate for assay (separately determine the water *<2.48>* in the same manner as Oxycodone Hydrochloride Hydrate) and about 0.1 g of hydrocotarnine hydrochloride hydrate 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 exactly 10 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. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate
($C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$)
$$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/25 \times 1.154$$

Amount (mg) of hydrocotarnine hydrochloride hydrate
($C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O$)
$$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/25 \times 1.070$$

M_{Sa}: Amount (mg) of oxycodone hydrochloride hydrate for assay taken, calculated on the anhydrous basis

M_{Sb}: Amount (mg) of hydrocotarnine hydrochloride hydrate for assay taken

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

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

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

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogen phosphate TS add 0.05 mol/L sodium dihydrogen phosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

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

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarnine in this order, with complete separation of these peaks.

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

Storage—Light-resistant.

Compound Oxycodone and Atropine Injection

Hycoato Injection

複方オキシコドン・アトロピン注射液

Compound Oxycodone and Atropine Injection is an aqueous injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$: 405.87), not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O$: 275.73), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [($C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$: 694.83].

Method of preparation

Oxycodone Hydrochloride Hydrate	8 g
Hydrocotarnine Hydrochloride Hydrate	2 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingre-

dients.

Description   Compound Oxycodone and Atropine Injection is a colorless or pale yellow, clear liquid.

It is affected by light.

pH: 2.5 – 4.0

Identification   (1)   To 1 mL of Compound Oxycodone and Atropin Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2)   Evaporate 1 mL of Compound Oxycodone and Atropin Injection on a water bath, and dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3)   Evaporate 1 mL of Compound Oxycodone and Atropin Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

(4)   To 1 mL of Compound Oxycodone and Atropine Injection add 0.5 mL of 2,4-dinitrophenylhydrazine-ethanol TS, and allow to stand for 1 hour. Centrifuge, and add acetone to the supernatant liquid until no more precipitate is produced. Allow to stand for 20 minutes, and centrifuge. To the supernatant liquid add potassium hydroxide TS until the liquid is light purple. Shake the liquid with 5 mL of dichloromethane, and separate the dichloromethane layer. Take 0.5 mL of the dichloromethane layer, and evaporate to dryness on a water bath. Add 5 drops of fuming nitric acid to the residue, and evaporate to dryness on a water bath. Cool, dissolve the residue in 1 mL of N,N-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced (atropine).

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

Assay   (1)   Oxycodone hydrochloride hydrate and hydrocotarnine hydrochloride hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride hydrate for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Oxycodone Hydrochloride Hydrate) and about 0.1 g of hydrocotarnine hydrochloride hydrate 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 exactly 10 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. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate
($C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$)
$$= M_{Sa} \times Q_{Ta}/Q_{Sa} \times 1/25 \times 1.154$$

Amount (mg) of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O$)
$$= M_{Sb} \times Q_{Tb}/Q_{Sb} \times 1/25 \times 1.070$$

M_{Sa}: Amount (mg) of oxycodone hydrochloride hydrate for assay taken, calculated on the anhydrous basis

M_{Sb}: Amount (mg) of hydrocotarnine hydrochloride hydrate for assay taken

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

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

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

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L sodium dihydrogenphosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

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

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarine in this order with complete separation of these peaks.

(2) Atropine sulfate hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10) and 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane layer through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate RS (separately determine the loss on drying <2.41> under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use so obtained solution as the standard solution. 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 atropine to that of the internal standards.

Amount (mg) of atropine sulfate hydrate
[$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$]
= $M_S \times Q_T/Q_S \times 1/50 \times 1.027$

M_S: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 1.5 m in length, packed with 180- to 250-μm siliceous earth for gas chromatography coated with 1 to 3% of 50% phenyl-methylsilicone polymer.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

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

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and atropine in this order with the resolution between these peaks being not less than 3.

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

Storage—Light-resistant.

Oxydol

オキシドール

Oxydol contains not less than 2.5 w/v% and not more than 3.5 w/v% of hydrogen peroxide (H_2O_2: 34.01). It contains suitable stabilizers.

Description Oxydol occurs as a clear, colorless liquid. It is odorless or has an odor resembling that of ozone.

It gradually decomposes upon standing or upon vigorous agitation.

It rapidly decomposes when in contact with oxidizing substances as well as reducing substances.

It, when alkalized, decomposes with effervescence.

It is affected by light.

pH: 3.0 – 5.0

Specific gravity d_{20}^{20}: about 1.01

Identification 1 mL of Oxydol responds to the Qualitative Tests <1.09> for peroxide.

Purity **(1)** Acidity—To 25.0 mL of Oxydol add 2 drops of phenolphthalein TS and 2.5 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <1.07>—To 5.0 mL of Oxydol add 20 mL of water and 2 mL of ammonia TS, evaporate on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid by heating, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(3) Arsenic <1.11>—To 1.0 mL of Oxydol add 1 mL of ammonia TS, evaporate on a water bath to dryness, take the residue, prepare the test solution according to Method 1, and perform the test (not more than 2 ppm).

(4) Organic stabilizer—Extract 100 mL of Oxydol with 50-mL, 25-mL and 25-mL portions of a mixture of chloroform and diethyl ether (3:2) successively, combine the extracts in a tared vessel, and evaporate the combined extract on a water bath. Dry the residue over silica gel to constant mass: the mass of the residue is not more than 50 mg.

(5) Nonvolatile residue—Evaporate 20.0 mL of Oxydol on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 20 mg.

Assay Pipet 1.0 mL of Oxydol, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate <2.50> with 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS
= 1.701 mg of H_2O_2

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 30°C.

Oxygen

酸素

O$_2$: 32.00

Oxygen is oxygen produced by the air liquification separation method.

It contains not less than 99.5 v/v% of oxygen (O$_2$).

Description　Oxygen is a colorless gas under atmospheric pressure, and is odorless.

1 mL of Oxygen dissolves in 32 mL of water, and in 7 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

1000 mL of Oxygen at 0°C and at a pressure of 101.3 kPa weighs 1.429 g.

Identification　Transfer 1 mL each of Oxygen and oxygen directly from cylinders with a pressure-reducing valve to gas-measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <2.02> according to the following conditions: the retention time of principal peak in the chromatogram obtained from Oxygen is the same as that of the peak in the chromatogram obtained from oxygen.

Operating conditions—

Proceed as directed in the operating conditions in the Purity.

Purity　Nitrogen—Transfer 1.0 mL of Oxygen directly from cylinder with a pressure-reducing valve to gas-measuring tube or syringe for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with this gas as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A_T of nitrogen. Introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL, allow to mix thoroughly and use this gas as the standard mixed gas. Perform the test in the same manner with 1.0 mL of this mixture as directed above, and determine the peak area A_S of nitrogen: A_T is not larger than A_S.

Operating conditions—

Detector: A thermal conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with zeolite for gas chromatography 250- to 355-μm in particle diameter (a porosity of 0.5 nm).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

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

System suitability—

System performance: Introduce 0.5 mL of nitrogen into a gas mixer, add Oxygen to make 100 mL, and mix thoroughly. When the test is run with 1.0 mL of the mixture under the above operating conditions, oxygen and nitrogen are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 1.0 mL of the standard mixed gas under the above operating conditions, the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

Assay　(i)　Apparatus—The apparatus is shown diagrammatically in the accompanying figure. A is a 100-mL gas buret having a two-way stopcock a, b – c, d – e and e – f are

graduated in 0.1 mL, and c – d is graduated in 2 mL. A is properly connected with a leveling tube B by a thick rubber tube. Fill ammonium chloride-ammonia TS up to the middle of A and B. Place in the absorption ball g of the gas pipette C a coil of copper wire, not more than 2 mm in diameter, which extends to the uppermost portion of the bulb, add 125 mL of ammonium chloride-ammonia TS, and stopper with a rubber stopper i. Connect C with A using the thick rubber tube.
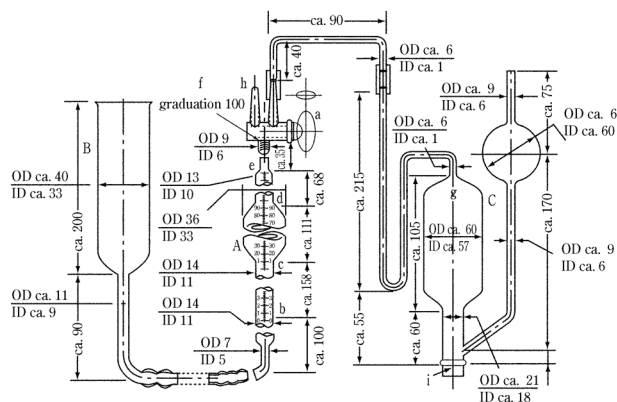
b–c: calibrated in 0.1 mL
c–d: calibrated in 2 mL
d–e: calibrated in 0.1 mL
e–f: calibrated in 0.1 mL
　The graduations are
　marked with red line.
b–f: =100 mL

(ii)　Procedure—Open a, set B downward and draw the liquid in g to the stopcock opening a. Then close a. Open a to the intake tube h, and fill A and h with ammonium chloride-ammonia TS by lifting B. Close a, connect h with a container of Oxygen, open a, set B downward and measure accurately 100 mL of Oxygen. Open a toward C, and transfer the Oxygen to g by lifting B. Close a, and rock C gently for 5 minutes. Open a, draw the residual gas back into A by setting B downward, and measure the volume of the residual gas. Repeat the procedure until the volume of residual gas is constant, and designate this as V (mL). With fresh ammonium chloride-ammonia TS in C, repeat the procedure at least four times, and measure the volume of residual gas. Calculate the volume of Oxygen and V in the following formula on the basis of the gas volume at 20°C and at 101.3 kPa.
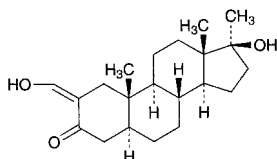
$$\text{Volume (mL) of oxygen (O}_2\text{)} = \text{volume of Oxygen (mL)} - V \text{ (mL)}$$

Containers and storage　Containers—Cylinders.

Storage—Not exceeding 40°C.

Oxymetholone

オキシメトロン

C$_{21}$H$_{32}$O$_3$: 332.48
17β-Hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one
[*434-07-1*]

Oxymetholone, when dried, contains not less than 97.0% and not more than 103.0% of oxymetholone (C$_{21}$H$_{32}$O$_3$).

Description Oxymetholone occurs as a white to pale yellowish white crystalline powder. It is odorless.

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

It is gradually colored and decomposed by light.

Identification **(1)** Dissolve 2 mg of Oxymetholone in 1 mL of ethanol (95), and add 1 drop of iron (III) chloride TS: a purple color develops.

(2) Dissolve 0.01 g of Oxymetholone in methanol to make 50 mL. To 5 mL of the solution add 5 mL of sodium hydroxide-methanol TS and methanol 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.

(3) Determine the infrared absorption spectrum of Oxymetholone 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> [α]$_D^{20}$: $+34 - +38°$ (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 175 – 182°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Oxymetholone in 25 mL of 1,4-dioxane: the solution is clear, and shows a colorless to pale yellow color.

(2) Related subslances—Dissolve 50 mg of Oxymetholone in 5 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 for thin-layer chromatography, and air-dry the spot. Develop immediately the plate with a mixture of toluene and ethanol (99.5) (49:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 100°C for 3 to 5 minutes: any spot other than the principal spot and starting point 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 1.0% (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 40 mg of Oxymetholone, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. To exactly measured 5 mL of this solution add 5 mL of sodium hydroxide-methanol TS and methanol to make exactly 50 mL. Determine the absorbance *A* of this solution at the wavelength of maximum absorption at about 315 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by adding methanol to 5 mL of sodium hydroxide-methanol TS to make 50 mL, as the blank.
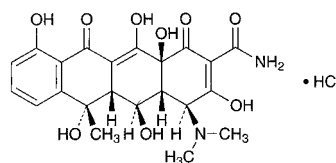
$$\text{Amount (mg) of oxymetholone (C}_{21}\text{H}_{32}\text{O}_3)$$
$$= A/541 \times 50{,}000$$

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

Oxytetracycline Hydrochloride

オキシテトラサイクリン塩酸塩

C$_{22}$H$_{24}$N$_2$O$_9$.HCl: 496.89
(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-Dimethylamino-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride
[*2058-46-0*]

Oxytetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces rimosus*.

It contains not less than 880 μg (potency) and not more than 945 μg (potency) per mg, calculated on the dried basis. The potency of Oxytetracycline Hydrochloride is expressed as mass (potency) of oxytetracycline (C$_{22}$H$_{24}$N$_2$O$_9$: 460.43).

Description Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride 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 or the spectrum of a solution of Oxytetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation <2.49> [α]$_D^{20}$: $-188 - -200°$ (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 0.5 g of Oxytetracycline Hydrochloride 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) Related substances—Dissolve 20 mg of Oxytetracycline Hydrochloride 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 4-epioxytetracycline in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 4-epioxytetracycline stock solution. Separately, dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as tetracycline hydrochloride stock solution. Separately, dissolve 8 mg of β-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as β-apooxytetracycline stock solution. Pipet 1 mL of 4-epioxytetracycline stock solution, 4 mL of tetracycline hydrochloride stock solution and 40 mL of β-apooxytetracycline stock 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 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 4-epioxytetracycline and tetracycline obtained from the sample solution are not larger than each of the peak area obtained from the standard solution, and the total area of the peaks, α-apooxytetracycline having the relative retention time of about 2.1 to oxytetracycline, β-apooxytetracycline and the peaks, which appear between α-apooxytetracycline and β-apooxytetracycline, is not larger than the peak area of β-apooxytetracycline from the standard solution. The peak area of 2-acetyl-2-decarboxamide oxytetracycline, which appears after the principal peak, obtained from the sample solution is not larger than 4 times the peak area of 4-epioxytetracycline 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 A: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 100 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 30 g of *t*-butyl alcohol and water to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 100 g of *t*-butyl alcohol and 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	70 → 10	30 → 90
20 – 35	10 → 20	90 → 80

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of 4-epioxytetracycline obtained from 20 μL of this solution is equivalent to 14 to 26% of that obtained from 20 μL of the standard solution.

System performance: Dissolve 8 mg of α-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as α-apooxytetracycline stock solution. Mix 3 mL of the sample solution, 2 mL of 4-epioxytetracycline stock solution, 6 mL of tetracycline hydrochloride stock solution, 6 mL of β-apooxytetracycline stock solution and 6 mL of α-apooxytetracycline stock solution, and 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, 4-epioxytetracycline, oxytetracycline, tetracycline, α-apooxytetracycline and β-apooxytetracycline are eluted in this order with the resolutions between the peaks, 4-epioxytetracycline and oxytetracycline, oxytetracycline and tetracycline, and α-apooxytetracycline and β-apooxytetracycline being not less than 4, not less than 5 and not less than 4, respectively, and the symmetry factor of the peak of oxytetracycline is not more than 1.3.

System repeatability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. 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 4-epioxytetracycline is not more than 2.0%.

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 0.5% (1 g).

Assay Weigh accurately an amount of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride RS, equivalent to about 50 mg (potency), and dissolve each in diluted hydrochloric acid (1 in 100) to make exactly 50 mL. Pipet 5 mL each of these solutions, add diluted methanol (3 in 20) to make exactly 50 mL, and use these solutions as the sample solution and 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 oxytetracycline in each solution.

Amount [μg (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$)
$$= M_S \times A_T/A_S \times 1000$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mL of methanol, and adjust the pH to 4.5 with dilute hydrochloric acid.

Flow rate: Adjust so that the retention time of oxytetracycline 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, the number of the theoretical plates and the symmetry factor of the peak of oxytetracycline are not less than 1000 and not more than 2.0, respectively.
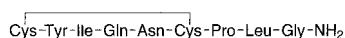
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.0%.

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

Oxytocin

オキシトシン

Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–Gly–NH₂

$C_{43}H_{66}N_{12}O_{12}S_2$: 1007.19
[*50-56-6*]

Oxytocin is a synthetic peptide having the property of causing the contraction of uterine smooth muscle.

It contains not less than 540 oxytocin Units and not more than 600 oxytocin Units per mg, calculated on the anhydrous and residual acetic acid-free basis.

Description   Oxytocin occurs as a white powder.

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

It dissolves in hydrochloric acid TS.

The pH of a solution prepared by dissolving 0.10 g of Oxytocin in 10 mL of freshly boiled and cooled water is between 4.0 and 6.0.

It is hygroscopic.

Identification   Determine the absorption spectrum of a solution of Oxytocin (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.

Constituent amino acids   Put about 1 mg of Oxytocin in a test tube for hydrolysis, add 6 mol/L hydrochloric acid TS to dissolve, replace the air in the tube with Nitrogen, seal the tube under reduced pressure, and heat at 110 to 115°C for 16 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, add 2 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-

glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add 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 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 respective molar ratios with respect to leucine: 0.95 – 1.05 for aspartic acid, 0.95 – 1.05 for glutamic acid, 0.95 – 1.05 for proline, 0.95 – 1.05 for glycine, 0.80 – 1.10 for isoleucine, 0.80 – 1.05 for tyrosine and 0.80 – 1.05 for cystine, and not more than 0.01 each for others.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 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 copolymer (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.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B and C according to the following table.

Mobile phase	A	B	C
Citric acid mono-hydrate	19.80 g	22.00 g	6.10 g
Trisodium citrate dihydrate	6.19 g	7.74 g	26.67 g
Sodium chloride	5.66 g	7.07 g	54.35 g
Ethanol (99.5)	260.0 mL	20.0 mL	—
Benzyl alcohol	—	—	5.0 mL
Thiodiglycol	5.0 mL	5.0 mL	—
Lauromacrogol solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL
Capryric acid	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	2000 mL	1000 mL	1000 mL

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)
0 – 9	100	0	0
9 – 25	0	100	0
25 – 61	0	100 → 0	0 → 100
61 – 80	0	0	100

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 more than 10 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 more than 30 minutes while passing Nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Flow rate of mobile phase: About 0.26 mL per minute.

Flow rate of reaction reagent: About 0.3 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.5, 1.4 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 20 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 2.0%, respectively.

Purity **(1)** Acetic acid—Weigh accurately about 15 mg of Oxytocin, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), add the internal standard solution to make exactly 100 mL. Pipet 2 mL of this solution, add the internal standard solution to make exactly 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 acetic acid to that of the internal standard: the amount of acetic acid is not less than 6.0% and not more than 10.0%.

$$\text{Amount (\%) of acetic acid (C}_2\text{H}_4\text{O}_2)}$$
$$= M_S/M_T \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of acetic acid (100) taken
M_T: Amount (mg) of Oxytocin taken

*Internal standard solution—*A solution of propionic acid in the mobile phase (1 in 10,000).

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 40°C.

Mobile phase: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust so that the retention time of acetic acid 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, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 14.

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 acetic acid to that of the internal standard is not more than 2.0%.

(2) Related substances—Dissolve 25 mg of Oxytocin in 100 mL of the mobile phase A, and use this solution as the sample solution. Perform the test with 50 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 amount of each peak other than Oxytocin is not more than 1.5%, and the total of them is not more than 5.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, flowing of 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 oxytocin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase A 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 the mobile phase A to make exactly 10 mL. Confirm that the peak area of oxytocin obtained from 50 μL of this solution is equivalent to 5 to 15% of that obtained from 50 μL of the solution for system suitability test.

System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 50 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

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 oxytocin is not more than 2.0%.

Water <2.48> Not more than 5.0% (50 mg, coulometric titration).

Assay Weigh accurately an amount of Oxytocin, equivalent to about 13,000 Units, dissolve in the mobile phase A to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 1 bottle of the Oxytocin RS in the mobile phase A to make a known concentration solution containing each mL contains about 130 Units, and use this solution as the standard solution. Perform the test with exactly 25 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 oxytocin in each solution.

Units per mg of Oxytocin, calculated on the anhydrous and residual acetic acid-free basis
$$= M_S/M_T \times A_T/A_S \times 100$$

M_S: Units per mL of the standard solution
M_T: Amount (mg) of Oxytocin taken, calculated on the anhydrous and residual acetic acid-free 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 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water 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 – 30	70 → 40	30 → 60
30 – 30.1	40 → 70	60 → 30
30.1 – 45	70	30

Flow rate: 1.0 mL per minute.
System suitability—

System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 25 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 1.0%.

Containers and storage   Containers—Tight containers.
Storage—At 2 to 8°C.

Oxytocin Injection

オキシトシン注射液

Oxytocin Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled oxytocin Units.

Method of preparation   Prepare as directed under Injections, with Oxytocin.

Description   Oxytocin Injection is a colorless, clear liquid.

pH *‹2.54›*   2.5 – 4.5

Bacterial endotoxins *‹4.01›*   Less than 10 EU/oxytocin Unit.

Extractable volume *‹6.05›*   It meets the requirement.

Foreign insoluble matter *‹6.06›*   Perform the test according to the 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 portion of Oxytocin Injection according to the labeled Units, dilute with the diluent so that each mL contains about 1 Unit, and use this solution as the sample solution. Separately, dissolve 1 bottle of Oxytocin RS in the mobile phase A to make exactly 20 mL. Pipet a suitable volume of this solution, dilute with the diluent to make a known concentration solution so that each mL contains

about 1 Unit, 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 oxytocin in each solution.

$$\text{Units per mL of Oxytocin Injection} = M_S \times A_T/A_S \times b/a$$

M_S: Units per mL of the standard solution
a: Volume (mL) of Oxytocin Injection taken
b: Total volume of the sample solution prepared by diluting with the diluent
Diluent: Dissolve 5 g of chlorobutanol, 1.1 g of sodium acetate trihydrate, 5 g of acetic acid (100) and 6 mL of ethanol (99.5) in water to make 1000 mL.

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 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water 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 – 30	70 → 40	30 → 60
30 – 30.1	40 → 70	60 → 30
30.1 – 45	70	30

Flow rate: 1.0 mL per minute.
System suitability—

System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.02 mg each of them. When the procedure is run with 100 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.
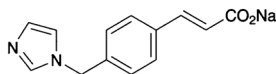
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

Containers and storage   Containers—Hermetic containers.
Storage—In a cold place, and avoid freezing.

Ozagrel Sodium

オザグレルナトリウム

C$_{13}$H$_{11}$N$_2$NaO$_2$: 250.23
Monosodium (2*E*)-3-[4-(1*H*-imidazol-1-ylmethyl)phenyl]prop-2-enoate
[*189224-26-8*]

Ozagrel Sodium, when dried, contains not less than 98.0% and not more than 102.0% of ozagrel sodium (C$_{13}$H$_{11}$N$_2$NaO$_2$).

Description   Ozagrel Sodium occurs as white, crystals or crystalline powder.

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

Identification   **(1)**   Determine the absorption spectrum of a solution of Ozagrel Sodium (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 Ozagrel 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 Ozagrel Sodium 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 Ozagrel Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)   A solution of Ozagrel Sodium (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt.

pH <2.54>   The pH of a solution prepared by dissolving 0.5 g of Ozagrel Sodium in 10 mL of water is between 9.5 and 10.5.

Purity   **(1)**   Clarity and color of solution—Dissolve 0.5 g of Ozagrel Sodium in 10 mL of water: the solution is clear and colorless.

(2)   Chloride <1.03>—Dissolve 2.0 g of Ozagrel Sodium in 30 mL of water, add 1 mL of acetic acid (100) and water to make 50 mL, shake, and allow to stand for 30 minutes. Filter the solution, discard the first 5 mL of the filtrate, and to 25 mL of the subsequent filtrate 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: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(3)   Heavy metals <1.07>—Proceed with 2.0 g of Ozagrel 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).

(4)   Related substances—Dissolve 50 mg of Ozagrel Sodium in 100 mL of the mobile phase, 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 each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: each of the amount other than ozagrel is not more than 0.2%, and the total amount other than ozagrel 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: 220 nm).

Time span of measurement: About 2 times as long as the retention time of ozagrel, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 200 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 to make exactly 10 mL. Confirm that the peak area of ozagrel obtained from 5 μL of this solution is equivalent to 15 to 25% of that obtained from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 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 ozagrel are not less than 6000 and not more than 2.0, 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 ozagrel is not more than 2.0%.

Loss on drying <2.41>   Not more than 0.5% (1 g, 105°C, 4 hours).

Assay   Weigh accurately about 25 mg each of Ozagrel Sodium and Ozagrel Sodium RS, both previously dried, and dissolve each in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 1 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 ozagrel to that of the internal standard.

$$\text{Amount (mg) of ozagrel sodium (C}_{13}\text{H}_{11}\text{N}_2\text{NaO}_2)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Ozagrel Sodium RS taken

*Internal standard solution—*A solution of benzoic acid in methanol (1 in 100).
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 25°C.

Mobile phase: A mixture of a solution of ammonium acetate (3 in 1000) and methanol (4:1).

Flow rate: Adjust so that the retention time of ozagrel is about 10 minutes.
System suitability—
System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the internal standard and ozagrel 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 ozagrel is not more 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 ratio of the peak area of ozagrel to that of the internal standard is not more than 1.0%.

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

Ozagrel Sodium Injection

オザグレルナトリウム注射液

Ozagrel Sodium Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$: 250.23).

Method of preparation Prepare as directed under Injections, with Ozagrel Sodium.

Description Ozagrel Sodium Injection occurs as a clear and colorless liquid.

Identification To a suitable volume of Ozagrel Sodium Injection add water so that each mL contains 5 μg of Ozagrel Sodium. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 269 nm and 273 nm.

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

Purity Related substance—To a suitable volume of Ozagrel Sodium Injection add the mobile phase so that each mL contains 0.4 mg of Ozagrel Sodium, and use this solution as the sample solution. Then, proceed as directed in the Purity (4) under Ozagrel Sodium.

Bacterial endotoxins <4.01> Less than 3.7 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 Perform the test following the test 1). The test 2) may be performed instead of 1), if possible.
1) To exactly a volume of Ozagrel Sodium Injection, equivalent to about 4 mg of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$), add exactly 5 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Ozagrel Sodium RS, 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 5 mL of the internal standard solution, add 10 mL of water, then add methanol to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ozagrel Sodium.

$$\text{Amount (mg) of ozagrel sodium } (C_{13}H_{11}N_2NaO_2)$$
$$= M_S \times Q_T / Q_S \times 1/10$$

M_S: Amount (mg) of Ozagrel Sodium RS taken

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

2) To exactly a volume of Ozagrel Sodium Injection, equivalent to about 20 mg of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$) add water to make exactly 10 mL. Pipet 1 mL of this solution, add exactly 2 mL of the internal standard solution, add 1 mL of water, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ozagrel Sodium RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ozagrel Sodium.

$$\text{Amount (mg) of ozagrel sodium } (C_{13}H_{11}N_2NaO_2)$$
$$= M_S \times Q_T / Q_S \times 4/5$$

M_S: Amount (mg) of Ozagrel Sodium RS taken

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used. Storage—Light-resistant.

Ozagrel Sodium for Injection

注射用オザグレルナトリウム

Ozagrel Sodium for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$: 250.23).

Method of preparation Prepare as directed under Injections, with Ozagrel Sodium.

Description Ozagrel Sodium for Injection occurs as white, masses or powder.

Identification Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 40 mg of Ozagrel Sodium, in water to make 40 mL. To 1 mL of this solution add water to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 269 nm and 273 nm.

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

Purity Related substances—Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 0.20 g of Ozagrel Sodium, in the mobile phase to make 100 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Purity (4) under Ozagrel Sodium.

Bacterial endotoxins <4.01> Less than 3.7 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 Ozagrel Sodium for Injection,

equivalent to about 0.4 g of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$), and dissolve all the contents in water to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and 5 mL of water, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ozagrel Sodium RS, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 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 under Ozagrel Sodium.

Amount (mg) of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$)
$$= M_S \times Q_T/Q_S \times 16$$

M_S: Amount (mg) of Ozagrel Sodium RS taken

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

Containers and storage Containers—Hermetic containers.

Pancreatin

パンクレアチン

Pancreatin is a substance containing enzymes prepared from the pancreas of edible animals, mostly the hog, and has amylolytic, proteolytic and lipolytic activities.

It contains not less than 2800 starch saccharifying activity units, not less than 28,000 proteolytic activity units, and not less than 960 lipolytic activity units per g.

It is usually diluted with suitable excipients.

Description Pancreatin occurs as a white to light yellow powder. It has a characteristic odor.

Purity (1) Rancidity—Pancreatin has no unpleasant or rancid odor and is tasteless.

(2) Fat—Add 20 mL of diethyl ether to 1.0 g of Pancreatin, 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 does not exceed 20 mg.

Loss on drying <2.41> Not more than 4.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <2.44> Not more than 5% (1 g).

Assay (1) Starch digestive activity <4.03>

(i) Substrate solution—Use potato starch TS for amylolytic activity test, prepared by adding 10 mL of phosphate buffer solution for pancreatin instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution (pH 5.0).

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL. Pipet 10 mL of this solution, and add ice-cold 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.

(2) Protein digestive activity <4.03>

(i) Substrate solution—Use the substrate solution 2 described in 2.3. (ii) of 2. Assay for protein digestive activity under Digestion Test after adjusting the pH to 8.5.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 200 mL.

(iii) Procedure—Proceed as directed in 2. Assay for protein digestive activity under Digestion Test, using trichloroacetic acid TS B as the precipitation reagent.

(3) Fat digestive activity <4.03>

(i) Emulsifier—Prepare with 18 g of polyvinyl alcohol I and 2 g of polyvinyl alcohol II as directed in 3. Assay for fat digestive activity under Digestion Test.

(ii) Substrate solution—Use the substrate solution described in 3. Assay for fat digestive activity under the Digestion Test.

(iii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL.
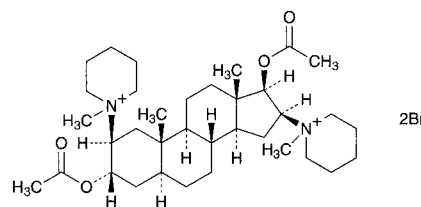
(iv) Procedure—Proceed as directed in 3. Assay for fat digestive activity under Digestion Test, using phosphate buffer solution (pH 8.0) as the buffer solution.

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Pancuronium Bromide

パンクロニウム臭化物

$C_{35}H_{60}Br_2N_2O_4$: 732.67
1,1′-(3α,17β-Diacetoxy-5α-androstan-2β,16β-diyl)bis(1-methylpiperidinium) dibromide
[*15500-66-0*]

Pancuronium Bromide contains not less than 98.0% and not more than 102.0% of pancuronium bromide ($C_{35}H_{60}Br_2N_2O_4$), calculated on the anhydrous basis.

Description Pancuronium Bromide occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95) and in acetic anhydride.

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Pancuronium 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.

(2) A solution of Pancuronium Bromide (1 in 100) responds to the Qualitative Tests <1.09> (1) for bromide.

Optical rotation <2.49> $[\alpha]_D^{20}$: +38 – +42° (0.75 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution of Pancuronium Bromide (1 in 100) is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pancuronium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 50 mg of Pancuronium Bromide 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 (1). Separately, weigh exactly 5 mg of dacuronium bromide for thin-layer chromatography, add ethanol (95) 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 2 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 2-propanol, acetonitrile and a solution of sodium iodide (1 in 5) (17:2:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of sodium nitrite in methanol (1 in 100) on the plate, allow to stand for 2 minutes, and spray evenly potassium bismuth iodide TS on the plate: a spot from the sample solution, corresponding to that from the standard solution (2), has no more color than that from the standard solution (2), and the spots other than the principal spot and the above mentioned spot from the sample solution have no more color than the spot from the standard solution (1).

Water <2.48>   Not more than 8.0% (0.3 g, volumetric titration, direct titration).

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

Assay   Weigh accurately about 0.2 g of Pancuronium Bromide, dissolve in 50 mL of acetic anhydride by warming, 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
= 36.63 mg of $C_{35}H_{60}Br_2N_2O_4$

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

Panipenem

パニペネム
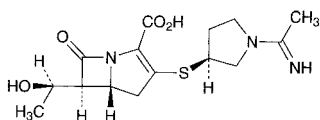
$C_{15}H_{21}N_3O_4S$: 339.41
(5R,6S)-6-[(1R)-1-Hydroxyethyl]-3-[(3S)-1-(1-iminoethyl)pyrrolidin-3-ylsulfanyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid
[*87726-17-8*]

Panipenem contains not less than 900 µg (potency) and not more than 1010 µg (potency) per mg, calculated on the anhydrous and residual solvent-free basis. The potency of Panipenem is expressed as mass (potency) of panipenem ($C_{15}H_{21}N_3O_4S$).

Description   Panipenem occurs as a white to light yellow, crystalline powder or mass.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is hygroscopic.

It deliquesces in the presence of moisture.

Identification   (1)   Dissolve 20 mg of Panipenem in 2 mL of water, add 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 color develops.

(2)   Determine the absorption spectrum of a solution of Panipenem in 0.02 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: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3)   Determine the infrared absorption spectrum of Panipenem 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> $[\alpha]_D^{20}$: +55 – +65° (0.1 g calculated on the anhydrous and residual solvent-free basis, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0), 10 mL, 100 mm).

pH <2.54>   Dissolve 0.5 g of Panipenem in 10 mL of water: the pH of the solution is between 4.5 and 6.5.

Purity   (1)   Clarity and color of solution—Dissolve 0.30 g of Panipenem in 40 mL of water, and observe immediately: the solution is clear and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.4.

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Panipenem 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—Keep the sample solution at 5°C or below. Dissolve 50 mg of Panipenem in 50 mL of water, 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 amount of them by the area percentage method: the amount of the peak other than panipenem is not more than 2.0%, and the total amount of the peaks other than panipenem is not more than 6.0%.
Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized porous glass for liquid chromatography (7 µm in particle diameter).

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

Mobile phase A: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in 700 mL of water, adjust to pH 8.0 with dilute sodium hydroxide TS, then add water to make 1000 mL, and add 20 mL of acetonitrile.

Mobile phase B: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in 700 mL of water, adjust to pH 8.0 with dilute sodium hydroxide TS, then add water to make 1000 mL. To 750 mL of this solution add 250 mL of 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 – 15	100	0
15 – 50	100 → 0	0 → 100

Flow rate: 1.0 mL per minute (the retention time of panipenem is about 16 minutes).

Time span of measurement: For 50 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Use a solution of Panipenem (1 in 100,000) as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add water to make exactly 10 mL. Confirm that the peak area of panipenem obtained with 10 μL of this solution is equivalent to 7 to 13% 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 conditions, the number of theoretical plates and the symmetry factor of the peak of panipenem 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 solution for system suitability test under the above conditions, the relative standard deviation of the peak area of panipenem is not more than 2.0%.

Water Weigh accurately about 0.5 g of Panipenem, transfer to a 15-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, weigh accurately 2 g of water, and add the internal standard solution to make exactly 100 mL. Pipet 5 mL and 10 mL of this solution, add the internal standard solution to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with 1 μL of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <2.02> according to the following condition, and calculate the ratios, Q_T, Q_{S1} and Q_{S2} of the peak area of water to that of the internal standard. Calculate the amount of water by the following formula: water is not more than 5.0%.

Amount of water (%)
$$= M_S/M_T \times (Q_T + Q_{S2} - 2Q_{S1})/2(Q_{S2} - Q_{S1}) \times 1/100 \times 100$$

M_S: Amount (g) of water taken
M_T: Amount (g) of Panipenem taken

Internal standard solution—A solution of acetonitrile in methanol (1 in 100).

Operating conditions—

Detector: A thermal conductivity detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous ethyl vinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 125°C.

Carrier gas: Helium.

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

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, water, methanol, and the internal standard are eluted in this order with the resolution between the peaks of water and internal standard being not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of water to that of the internal standard is not more than 5.0%.

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Conduct this procedure within 30 minutes after preparation of the sample and standard solutions. Weigh accurately an amount of Panipenem and Panipenem RS, equivalent to about 0.1 g (potency), dissolve them separately in 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 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 panipenem to that of the internal standard.

Amount [μg (potency)] of panipenem ($C_{15}H_{21}N_3O_4S$)
$$= M_S \times Q_T/Q_S \times 1000$$

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

Internal standard solution—A solution of sodium *p*-styrenesulfonate in 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution (pH 7.0) (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 25 cm in length, packed with octadecylsilanized silicone polymer coated silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution (pH 8.0) and acetonitrile (50:1).

Flow rate: Adjust so that the retention time of the internal standard 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, panipenem 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 ratios of the peak area of panipenem to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—At a temperature not exceeding −10°C.

Panipenem and Betamipron for Injection

注射用パニペネム・ベタミプロン

Panipenem and Betamipron for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 105.0% of the labeled potency of panipenem ($C_{15}H_{21}N_3O_4S$: 339.41), and not less than 95.0% and not more than 105.0% of labeled amount of betamipron ($C_{10}H_{11}NO_3$: 193.20).

Method of preparation Prepare as directed under Injections, with Panipenem and Betamipron.

Description Panipenem and Betamipron for Injection occurs as two layers of upper and lower. The former occurs pale yellowish white to light yellow, masses or masses containing powder, and the latter white, masses or masses containing powder.

It is deliquescent.

Identification (1) Powder Panipenem and Betamipron for Injection, weigh a portion of the powder, equivalent to 40 mg (potency) of Panipenem, dissolve in 4 mL of water, add 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 color develops (panipenem).

(2) Powder Panipenem and Betamipron for Injection. Dissolve a portion of the powder, equivalent to 50 mg of Betamipron, in 4 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Separately, dissolve 12 mg of betamipron in 1 mL of diluted methanol (1 in 2), 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5) and triethylamine (19:1) to a distance of about 8 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 (betamipron).

pH <2.54> The pH of a solution of an amount of Panipenem and Betamipron for Injection, equivalent to 0.5 mg (potency) of Panipenem, in 100 mL of isotonic sodium chloride solution is 5.8 to 7.8.

Purity (1) Clarity and color of solution—A solution of an amount of Panipenem and Betamipron for Injection, equivalent to 0.5 g (potency) of Panipenem, in 10 mL of water is clear, and has no more color than Matching Fluid J.

(2) Related substances—After preparation of the sample solution, keep it at not exceeding 5°C and use within 60 minutes. Take 1 container of Panipenem and Betamipron for Injection, dissolve in water so that each mL contains 1 mg (potency) of panipenem, 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, and 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 other than panipenem and betamipron

is not more than 8.0%, and the total amount of peaks other than panipenem and betamipron is not more than 13.0%.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter, 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: A mixture of 0.02 mol/L phosphate buffer (pH 8.0) and acetonitrile (100:1).

Mobile phase B: A mixture of 0.02 mol/L phosphate buffer (pH 8.0) and acetonitrile (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 – 22	100	0
22 – 25	100 → 90	0 → 10
25 – 30	90	10
30 – 35	90 → 85	10 → 15
35 – 40	85 → 77	15 → 23
40 – 50	77 → 0	23 → 100
50 – 55	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3 times as long as the retention time of panipenem.

System suitability—

Test for required detectability: Use the diluted sample solution (1 in 100) as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add water to make exactly 10 mL. Confirm that the peak area of panipenem obtained with 10 μL of this solution is equivalent to 7 to 13% 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 panipenem are not less than 4000 and 0.8 to 1.2, respectively.

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 panipenem is not more than 0.95%.

Bacterial endotoxins <4.01> Less than 0.15 EU/mg (potency).

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

After preparation of the sample solution and standard solution, keep them at not exceeding 5°C. Dissolve the content of 1 container of Panipenem and Betamipron for Injection in 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 500 mL. Take exactly V mL of this solution, equivalent to 5 mg (potency) of Panipenem, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of panipenem ($C_{15}H_{21}N_3O_4S$)
    $= M_{S1} \times Q_{T1}/Q_{S1} \times 25/V$

Amount (mg) of betamipron ($C_{10}H_{11}NO_3$)
    $= M_{S2} \times Q_{T2}/Q_{S2} \times 25/V$

M_{S1}: Amount [mg (potency)] of Panipenem RS taken
M_{S2}: Amount (mg) of betamipron for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of sodium *p*-styrenesulfonate in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) (1 in 10,000).

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 After preparation of the sample solution and standard solution, keep them at not exceeding 5°C. Dissolve the total amount of the contents of 10 containers of Panipenem and Betamipron for Injection in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 500 mL. Take exactly *V* mL of this solution, equivalent to about 50 mg (potency) of Panipenem, add 0.02 mol/L 3-(*N*-morpholino)-propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Panipenem RS and about 50 mg of betamipron for assay (separately determine the water <2.48> in the same manner as Betamipron), dissolve in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) 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_{T1} and Q_{T2}, of the peak areas of panipenem and betamipron to that of the internal standard obtained from the sample solution, and the ratios, Q_{S1}, and Q_{S2}, of the peak areas of panipenem and betamipron to that of the internal standard obtained from the standard solution.

Amount [mg (potency)] of panipenem ($C_{15}H_{21}N_3O_4S$)
    $= M_{S1} \times Q_{T1}/Q_{S1} \times 25/V$

Amount (mg) of betamipron ($C_{10}H_{11}NO_3$)
    $= M_{S2} \times Q_{T2}/Q_{S2} \times 25/V$

M_{S1}: Amount [mg (potency)] of Panipenem RS taken
M_{S2}: Amount (mg) of betamipron for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of sodium *p*-styrenesulfonate in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution (pH 7.0) (1 in 10,000).
Operating conditions—
    Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay under Panipenem.
    Detector: An ultraviolet absorption photometer (wave-

length: 260 nm).
    Flow rate: Adjust so that the retention time of panipenem 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, betamipron, panipenem and the internal standard are eluted in this order, and the resolutions between the peaks of betamipron and panipenem, and panipenem and the internal standard are not less than 3, 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 ratios of the peak area of betamipron and panipenem to that of the internal standard are not more than 1.0%, respectively.

Containers and storage Containers—Hermetic containers.

Shelf life 24 months after preparation.

Pantethine

パンテチン
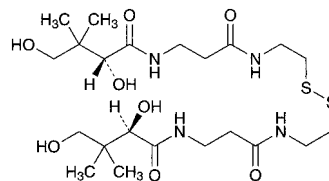
$C_{22}H_{42}N_4O_8S_2$: 554.72
Bis(2-{3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoylamino}ethyl) disulfide
[*16816-67-4*]

Pantethine is an aqueous solution containing 80% of pantethine.
Pantethine contains not less than 98.0% of pantethine ($C_{22}H_{42}N_4O_8S_2$), calculated on the anhydrous basis.

Description Pantethine is a clear, colorless to pale yellow viscous liquid.
It is miscible with water, with methanol and with ethanol (95).
It is decomposed by light.

Identification (1) To 0.7 g of Pantethine add 5 mL of sodium hydroxide TS, shake, and add 1 to 2 drops of copper (II) sulfate TS: a blue-purple color develops.

(2) To 0.7 g of Pantethine add 3 mL of water, shake, add 0.1 g of zinc powder and 2 mL of acetic acid (100), and boil for 2 to 3 minutes. After cooling, add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.

(3) To 1.0 g of Pantethine add 500 mL of water, and shake. To 5 mL of this solution add 3 mL of 1 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. After cooling, add 7 mL of a solution of hydroxylammonium chloride in sodium hydroxide TS (3 in 140), and allow to stand for 5 minutes. Add 3 drops of 2,4-dinitrophenol TS, and add 1 mol/L hydrochloric acid TS dropwise until the solution has no color, and then add 1 mL of iron (III) chloride TS: a red-purple color develops.

Optical rotation <2.49> $[\alpha]_D^{20}$: +15.0 – +18.0° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Pantethine 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) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Pantethine according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.6 g of Pantethine in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water 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 2-butanone saturated with water to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand for about 10 minutes in iodide vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Mercapto compounds—To 1.5 g of Pantethine add 20 mL of water, shake, add 1 drop of ammonia TS and 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red color is not developed.

Water <*2.48*> 18 – 22% (0.2 g, volumetric titration, direct titration).

Residue on Ignition <*2.44*> Not more than 0.1% (2 g).

Assay Weigh accurately about 0.3 g of Pantethine, add water to make exactly 20 mL. Transfer exactly 5 mL of this solution in an iodine bottle, and add exactly 25 mL of 0.05 mol/L bromine VS and 100 mL of water. Add 5 mL of diluted sulfuric acid (1 in 5) rapidly, stopper tightly immediately, and warm at 40 to 50°C for 15 minutes with occasional shaking. After cooling, carefully add 5 mL of a solution of potassium iodide (2 in 5), then immediately stopper tightly, shake, add 100 mL of water and titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.
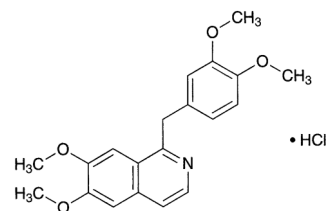
Each mL of 0.05 mol/L bromine VS
= 5.547 mg of $C_{22}H_{42}N_4O_8S_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, at a temperature not exceeding 10°C.

Papaverine Hydrochloride

パパベリン塩酸塩

$C_{20}H_{21}NO_4.HCl$: 375.85
6,7-Dimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline monohydrochloride
[*61-25-6*]

Papaverine Hydrochloride, when dried, contains not less than 98.5% of papaverine hydrochloride ($C_{20}H_{21}NO_4.HCl$).

Description Papaverine Hydrochloride occurs as white crystals or crystalline powder.

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

The pH of a solution of 1.0 g of Papaverine Hydrochloride in 50 mL of water is between 3.0 and 4.0.

Identification **(1)** To 1 mg of Papaverine Hydrochloride add 1 drops of formaldehyde-sulfuric acid TS: a colorless to light yellow-green color is produced, and it gradually changes to deep red, then to brown.

(2) Dissolve 0.02 g of Papaverine Hydrochloride in 1 mL of water, and add 3 drops of sodium acetate TS: a white precipitate is produced.

(3) Dissolve 1 mg of Papaverine Hydrochloride in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and examine under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(4) Dissolve 0.1 g of Papaverine Hydrochloride in 10 mL of water, make alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath, and dry the residue at 105°C for 3 hours: the residue so obtained melts <*2.60*> between 145°C and 148°C.

(5) Alkalify a solution of Papaverine Hydrochloride (1 in 50) with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests <*1.09*> (2) for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Papaverine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Papaverine Hydrochloride in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrate (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

(3) Readily carbonizable substances <*1.15*>—Perform the test with 0.12 g of Papaverine Hydrochloride: the solution has no more color than Matching Fluid S or P.

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 0.5 g of Papaverine Hydrochloride, previously dried, dissolve in 100 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.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 37.59 mg of $C_{20}H_{21}NO_4 \cdot HCl$

</div>

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

Papaverine Hydrochloride Injection

パパベリン塩酸塩注射液

Papaverine Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of papaverine hydrochloride ($C_{20}H_{21}NO_4 \cdot HCl$: 375.85).

Method of preparation Prepare as directed under Injections, with Papaverine Hydrochloride.

Description Papaverine Hydrochloride Injection is a clear, colorless liquid.
pH: 3.0 – 5.0

Identification (1) To 1 mL of Papaverine Hydrochloride Injection add 3 drops of sodium acetate TS: a white precipitate is produced.

(2) Dilute a volume of Papaverine Hydrochloride Injection, equivalent to 0.1 g of Papaverine Hydrochloride, with water to 10 mL, render the solution alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath to dryness, and dry the residue at 105°C for 3 hours: the residue so obtained melts <2.60> between 145°C and 148°C.

(3) Proceed with 1 mg each of the residue obtained in (2) as directed in the Identification (1) and (3) under Papaverine Hydrochloride.

(4) Alkalify 2 mL of Papaverine Hydrochloride Injection with ammonia TS, filter the precipitate off, and acidity the filtrate with dilute nitric acid: the solution responds to Qualitative Tests <1.09> (2) for chloride.

Bacterial endotoxins <4.01> Less than 6.0 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 Dilute an exactly measured volume of Papaverine Hydrochloride Injection, equivalent to about 0.2 g of papaverine hydrochloride ($C_{20}H_{21}NO_4 \cdot HCl$), with water to 10 mL, render the solution alkaline with ammonia TS, and extract with 20-mL, 15-mL, 10-mL and 10-mL portions of

chloroform. Combine the extracts, wash with 10 mL of water, and re-extract the washings with two 5-mL portions of chloroform. Combine all the chloroform extracts, and distil the chloroform on a water bath. Dissolve the residue in 30 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

<div align="center">

Each mL of 0.05 mol/L perchloric acid VS
= 18.79 mg of $C_{20}H_{21}NO_4 \cdot HCl$

</div>

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

Paraffin

パラフィン

Paraffin is a mixture of solid hydrocarbons obtained from petroleum.

Description Paraffin occurs as a colorless or white, more or less transparent, crystalline mass. It is odorless and tasteless.

It is sparingly soluble in diethyl ether and practically insoluble in water, in ethanol (95) and in ethanol (99.5).

Specific gravity d_{20}^{20}: about 0.92 (proceed as directed in 4.2. in 4. Specific gravity under Fats and Fatty Oils Test <1.13>).

Identification (1) Heat Paraffin strongly in a porcelain dish, and ignite: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 g of Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Melting point <2.60> 50 – 75°C (Method 2).

Purity (1) Acidity or alkalinity—Boil 10.0 g of Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS in a water bath for 5 minutes, and shake vigorously: a red color is not produced. Add 0.20 mL of 0.02 mol/L sodium hydroxide VS to this solution, and shake: a red color is produced.

(2) Heavy metals <1.07>—Ignite 2.0 g of Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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 and water to make 50 mL (not more than 10 ppm).

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

(4) Sulfur compounds—To 4.0 g of Paraffin add 2 mL of ethanol (99.5), further add 2 drops of a clear saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and heat for 10 minutes at 70°C with occasional shaking: no dark brown color develops in the aqueous layer.

(5) Readily carbonizable substances—Melt 5.0 g of Paraffin placed in a Nessler tube at a temperature near the melting point. Add 5 mL of sulfuric acid for readily carbonizable substances, and warm at 70°C for 5 minutes in a water bath. Remove the tube from the water bath, immediately shake vigorously and vertically for 3 seconds, and

warm for 1 minute in a water bath at 70°C. Repeat this procedure 5 times: the color of the sulfuric acid layer is not darker than that of the following control solution.

Control solution: Add 1.5 mL of Cobalt (II) Chloride CS, 0.5 mL of Copper (II) Sulfate CS and 5 mL of liquid paraffin to 3.0 mL of Iron (III) Chloride CS, and shake vigorously.

Containers and storage   Containers—Well-closed containers.

Liquid Paraffin

流動パラフィン

Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petrolatum.

Tocopherols of a suitable form may by added at a concentration not exceeding 0.001% as a stabilizer.

Description   Liquid Paraffin is a colorless, transparent, oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water and in ethanol (95).

Boiling point: above 300°C.

Identification   **(1)**   Heat Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2)   Heat 0.5 of Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity *<2.56>* d_{20}^{20}: 0.860 – 0.890

Viscosity *<2.53>*   Not less than 37 mm²/s (Method 1, 37.8°C).

Purity   **(1)**   Odor—Transfer a suitable amount of Liquid Paraffin to a small beaker, and heat on a water bath: a foreign odor is not perceptible.

(2)   Acidity or alkalinity—Shake vigorously 10 mL of Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3)   Heavy metals *<1.07>*—Ignite 2.0 g of Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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 and water to make 50 mL (not more than 10 ppm).

(4)   Arsenic *<1.11>*—Prepare the test solution with 1.0 g of Liquid Paraffin, according to Method 3 except that after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), add 1.5 mL of hydrogen peroxide (30), fire to burn, and perform the test (not more than 2 ppm).

(5)   Solid paraffin—Transfer 50 mL of Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6)   Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7)   Polycyclic aromatic hydrocarbons—Take 25 mL of Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution obtained as the sample solution. Transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to another 50-mL separator, shake vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution thus obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8)   Readily carbonizable substances—Transfer 5 mL of Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure 4 times: the Liquid Paraffin layer remains unchanged in color, and the sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS with 1.5 mL of Cobalt (II) Chloride CS and 0.50 mL of Copper (II) Sulfate CS.

Containers and storage   Containers—Tight containers.

Light Liquid Paraffin

軽質流動パラフィン

Light Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

Tocopherols of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

Description   Light Liquid Paraffin is a clear, colorless oily liquid, nearly free from fluorescence. It is odorless and tasteless.

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

Boiling point: above 300°C.

Identification   **(1)**   Heat Light Liquid Paraffin strongly in

a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Light Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity <2.56> d_{20}^{20}: 0.830 – 0.870

Viscosity <2.53> Less than 37 mm²/s (Method 1, 37.8°C).

Purity (1) Odor—Transfer a suitable amount of Light Liquid Paraffin to a small beaker, and heat on a water bath: no foreign odor is perceptible.

(2) Acidity or alkalinity—Shake vigorously 10 mL of Light Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Ignite 2.0 g of Light Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue 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 and water to make 50 mL (not more than 10 ppm).

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

(5) Solid paraffin—Transfer 50 mL of Light Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Light Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Light Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as the sample solution. Separately, transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to a 50-mL separator, add 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as a control

solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Light Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the liquid paraffin layer remains unchanged in color, and sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Iron (III) Chloride CS with 1.5 mL of Cobalt (II) Chloride CS and 0.50 mL of Copper (II) Sulfate CS.

Containers and storage Containers—Tight containers.

Paraformaldehyde

パラホルムアルデヒド

(CH₂O)ₙ
Poly(oxymethylene)
[30525-89-4]

Paraformaldehyde contains not less than 95.0% of formaldehyde (CH₂O: 30.03).

Description Paraformaldehyde occurs as a white powder. It has a slight odor of formaldehyde, but a very strong irritating odor is perceptible when it is heated.

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

It dissolves in hot water, in hot dilute hydrochloric acid, in sodium hydroxide TS and in ammonia TS.

It sublimes at about 100°C.

Identification (1) Dissolve 0.1 g of Paraformaldehyde in 5 mL of ammonia TS, add 5 mL of silver nitrate TS, shake, and add 3 mL of a solution of sodium hydroxide (1 in 10): a mirror of metallic silver is immediately formed on the sides of the container.

(2) Add a solution of 0.04 g of salicylic acid in 5 mL of sulfuric acid to 0.02 g of Paraformaldehyde, and warm slowly: a persistent, dark red color is produced.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Paraformaldehyde in 10 mL of ammonia TS: the solution is clear and colorless.

(2) Acidity or alkalinity—To 0.5 g of Paraformaldehyde add 10 mL of water, shake vigorously for 1 minute, and filter: the filtrate is neutral.

(3) Chloride <1.03>—Dissolve 1.5 g of Paraformaldehyde in 75 mL of water and 7.5 mL of sodium carbonate TS, evaporate 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 7.5 mL of sodium carbonate TS, a volume of diluted nitric acid (3 in 10) required for neutralization of the sample, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.006%).

(4) Sulfate <1.14>—Dissolve 1.5 g of Paraformaldehyde

in 45 mL of water and 4.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at abut 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize the diluted hydrochloric acid (3 in 5), and boil for 5 minutes. After cooling, 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 4.5 mL of sodium carbonate TS add an equal volume of diluted hydrochloric acid (3 in 5) for the neutralization of the sample and 15 mL of water, and boil for 5 minutes. After cooling, add 0.35 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.011%).

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

Assay   Dissolve about 50 mg of Paraformaldehyde, accurately weighed, in 10 mL of potassium hydroxide TS in an iodine flask. Add 40 mL of water and an exactly measured 50 mL of 0.05 mol/L iodine VS, stopper, and allow to stand for 5 minutes. Then add 5 mL of dilute hydrochloric acid, stopper immediately, allow to stand for 15 minutes, 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.

Dental Paraformaldehyde Paste

歯科用パラホルムパスタ

Method of preparation

Paraformaldehyde, finely powdered	35 g
Procaine Hydrochloride, finely powdered	35 g
Hydrous Lanolin	a sufficient quantity
	To make 100 g

Prepare as directed under Ointments, with the above ingredients.

Description   Dental Paraformaldehyde Paste is yellowish white in color. It has a characteristic odor.

Identification   (1)   To 0.15 g of Dental Paraformaldehyde Paste add 20 mL of diethyl ether and 20 mL of 0.5 mol/L sodium hydroxide TS, shake well, separate the water layer, and dilute with water to make 100 mL. To 1 mL of this solution add 10 mL of acetylacetone TS, and heat on a water bath for 10 minutes: a yellow color is produced (paraformaldehyde).

(2)   To the diethyl ether layer obtained in (1) add 5 mL of dilute hydrochloric acid and 20 mL of water, shake well, and separate the water layer: the solution responds to Qualitative Tests <1.09> for primary aromatic amines (procaine hydrochloride).

(3)   To 0.15 g of Dental Paraformaldehyde Paste add 25 mL of diethyl ether and 25 mL of water, shake, separate the water layer, filter, and use the filtrate as the sample solution. Seperately, dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as 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. Devel-

op 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. Examine under ultraviolet light (main wavelength: 254 nm): spots from the sample solution and standard solution show the same *R*f value.

Containers and storage   Containers—Tight containers.

Parnaparin Sodium

パルナパリンナトリウム

$R^1, R^3, R^4 = SO_3Na$ or H

$R^2 = SO_3Na$ or

$R^5 = CO_2Na$, $R^6 = H$

or

$R^5 = H$, $R^6 = CO_2Na$

n = 4 – 21
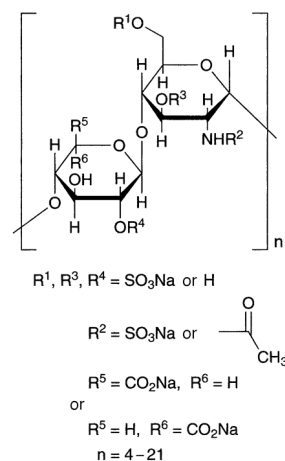
Parnaparin Sodium is a low-molecular heparin sodium obtained by depolymerization, with hydrogen peroxide and copper (II) acetate or with sodium hypochlorite, of heparins sodium from the healthy edible porcine intestinal mucosa. The mass-average molecular mass ranges between 4500 and 6500.

The potency is not less than 70 low-molecular-mass-heparin units and not more than 95 low-molecular-mass-heparin units of anti-factor Xa activity per mg, calculated on the dried basis.

Description   Parnaparin Sodium occurs as a white or light yellow powder.

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

It is hygroscopic.

Identification   (1)   Mix 0.1 mL of a solution of Parnaparin Sodium (1 in 20) and 10 mL of a solution of tritoluidine blue O (1 in 100,000), and shake the mixture: the blue color of solution immediately changes to purple.

(2)   A solution of Parnaparin Sodium (1 in 20) responds to Qualitative Tests <1.09> for sodium salt.

pH <2.54>   Dissolve 0.1 g of Parnaparin Sodium in 10 mL of water: the pH of this solution is between 6.0 and 8.0.

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Parnaparin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Parnaparin 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).

Loss on drying <2.41>   Not more than 8.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Molecular mass   Calculate the molecular mass of Parnaparin Sodium by the following methods: The mass-average molecular mass ranges between 4500 and 6500.

(i)   Creation of calibration curve—Weigh 20 mg of low-molecular mass heparin for calibration of molecular mass, and dissolve it in 2.0 mL of the mobile phase as the standard solution. Perform the test with 50 μL of the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak height, H_{UV}, in chromatogram obtained by the ultraviolet absorption photometer, and determine the peak height, H_{RI}, in chromatogram obtained by the differential refractometer. Calculate the ratio of H_{UV} to H_{RI}, H_{RI}/H_{UV}, at each peak. Assume the molecular mass in the 4th peak from the low molecular mass in chromatogram obtained by the ultraviolet absorption photometer as 2400, and make the calculation of the standard coefficient from dividing 2400 by the H_{RI}/H_{UV} at the corresponding peak. Make the calculation to multiply the H_{RI}/H_{UV} at each peak by the standard coefficient, and determine the molecular mass of each peak by the calculation. Prepare the calculation curve by plotting the logarithm of molecular masses at each peak on the vertical axis and the retention time on the chromatogram obtained by the differential refractometer on the horizontal axis.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm) and a differential refractometer.

Column: Connect 2 stainless steel columns which are 7.5 mm in inside diameter and 30 cm in length, and are packed with porous silica gel for liquid chromatography; one column, the molecular mass of limited size exclusion is about 500,000; the other, the molecular mass of limited size exclusion is about 100,000. Connect a pump, the about 500,000-molecular mass of limited size exclusion column, the about 100,000-molecular mass of limited size exclusion column, the ultraviolet absorption photometer and the differential refractometer in this order.

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

Mobile phase: Dissolve 28.4 g of sodium sulfate anhydride in 1000 mL of water, and 5.0 with 0.05 mol/L sulfuric acid TS.

Flow rate: 0.5 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, confirm that more than 10 peaks in chromatogram obtained as directed under either the Ultraviolet-visible Spectrophotometry, or the Differential Refractometry are observed.

System repeatability: When the tests repeated 6 times with 50 μL of the standard solution under the above operating conditions, relative standard deviation of the 4th peak height in chromatogram (H_{UV} and H_{RI}) is not more than 3.0%.

(ii)   Determination of molecular mass—Dissolve the 20 mg of Parnaparin Sodium with 2.0 mL of mobile phase, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Divide the main peak observed between 30 minutes and 45 minutes to 30 sec-interval fractions, and determine the strength of differential refractometer of each 30 sec-interval fraction. Determine the molecular mass of each fraction using the calibration curve and the retention time of each fraction. Determine the mean of molecular mass in the entire peak using the strength of differential refractometer and the molecular mass in every fractions.

Mean molecular mass of parnaparin sodium
$$= \Sigma(n_i \cdot M_i)/\Sigma n_i$$

n_i: The differential refractometer strength of fraction i in the main peak of chromatogram

M_i: Molecular mass of fraction i in main peak

Operating conditions—

Detector: A differential refractometer.

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in (i) Creation of calibration curve.

System suitability—

Proceed as directed in (i) Creation of calibration curve.

Distribution of molecular mass   The molecular mass of Parnaparin Sodium is calculated as directed in the determination of molecular mass and the distribution of molecular mass is calculated by the following equation: the molecular mass of not less than 80% parnaparin sodium is between 1500 and 10,000.

Distribution of molecular mass (%)
$$= (\Sigma n_j/\Sigma n_i) \times 100$$

n_i: The differential refractometer strength of fraction i in the main peak of chromatogram

Σn_j: Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

The degree of sulfate ester   Dissolve 0.5 g of Parnaparin Sodium with 10 mL water. Treat the solution with 5 mL of a strongly basic ion exchange resin, and subsequently with 10 mL of a strongly acidic ion exchange resin. Dilute the solution with water to 50 mL, and titrate <2.50> with 0.1 mol/L Sodium hydroxide VS (potentiometric titration). Calculate the degree of sulfate ester of Parnaparin Sodium from the equivalence point by the following equation; it is between 2.0 and 2.4.

The degree of sulfate ester
= the first equivalence point (mL)/[the second equivalence point (mL) – first equivalence point (mL)]

Total nitrogen   Weigh accurately about 0.10 g of Parnaparin Sodium which is dried, and perform the test as directed under Nitrogen Determination <1.08>: it contains not less than 1.9% and not more than 2.3% of nitrogen (N:14.01).

Anti-factor IIa activity   Determine the potency of anti-factor IIa activity of Parnaparin Sodium according to the following method, it contains not less than 35 and not more than 60 low-molecular-mass-heparin unit per mg, calculated on the dried basis.

(i)   Standard solution—Dissolve Low-molecular Mass Heparin RS with isotonic sodium chloride solution to make solutions which contain 0.1, 0.2 and 0.3 low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL, respectively.

(ii)   Sample solution—Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it with isotonic sodium chloride solution to adjust the solution which contains 4 μg parnaparin sodium in 1 mL.

(iii)   Procedure—To each plastic tube add 0.10 mL of the sample solution and the standard solution, separately. To each tube add 0.10 mL of human normal plasma and mix, and incubate at 37 ± 1°C accurately for 1 minute. Next, to each test tube add 0.10 mL of activated thromboplastin-time assay solution, which is pre-warmed at 37 ± 1°C, and after the mixing incubate accurately for 5 minutes at 37 ± 1°C. Then, to each tube add 0.10 mL of calcium chloride solution (277 in 100,000) which is pre-warmed at 37 ± 1°C, mix, start a stop watch simultaneously, and permit to stand at the

same temperature. Determine the time for the first appearance of fibrin clot.

(iv) Calculation—Determine the low-molecular-mass-heparin unit (anti-factor IIa activity) of the sample solution from calibration curve obtained plots of clotting times for each standard solution; calculate the low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium as following equation.

The low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium
= the low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL of sample solution × b/a

a: Amount (mg) of Parnaparin Sodium
b: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

The ratio of anti-factor Xa activity to anti-factor IIa activity Divide the anti-factor Xa activity, obtained in the Assay, by the anti-factor IIa activity which has been obtained from the test according to the method of anti-factor IIa activity; the ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

Assay

(i) Standard solution—Dissolve Low-molecular Mass-Heparin RS in isotonic sodium chloride solution to make solutions which contain 0.4, 0.6 and 0.8 low-molecular-mass-heparin units (anti-factor Xa activity) in 1 mL, respectively.

(ii) Sample solution—Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it in isotonic sodium chloride solution to make a solution which contains 7 μg parnaparin sodium in 1 mL.

(iii) Procedure—To each plastic tube add 0.10 mL of either the sample solution or the standard solution, separately. Subsequently to the every tubes add 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma, and mix them. To another plastic tube transfer 0.20 mL of these solutions, separately, and incubate for accurate 3 minutes at 37 ± 1°C. Next, to each tube add 0.10 mL of facter Xa TS and mix it, permit to stand 37 ± 1°C accurately for 30 seconds, and immediately add 0.20 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 3 min at 37 ± 1°C. To each test tube add 0.30 mL of diluted acetic acid (100) solution (1 in 2) to stop the reaction. Separately, to plastic tube add 0.10 mL of isotonic sodium chloride solution, 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III TS, and 0.10 mL of normal human plasma to every tubes, and mix well. To another plastic tube transfer 0.20 mL of the solution, separately, and add both 0.30 mL of water and 0.30 mL of diluted acetic acid (100) (1 in 2). Determine the absorbance of both the sample solution and the standard solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution obtained from this solution as the blank.

(iv) Calculation method—Determine the low-molecular-mass unit (anti-factor Xa activity) of the sample solution using the calibration curve prepared from the absorbance of the standard solutions and their logarithmic concentrations, and calculate the low-molecular-mass unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium.

Low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium
= the low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mL of the sample solution × b/a

a: Amount (mg) of Parnaparin Sodium taken
b: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution

Containers and storage Containers—Well-closed containers.

Paroxetine Hydrochloride Hydrate

パロキセチン塩酸塩水和物
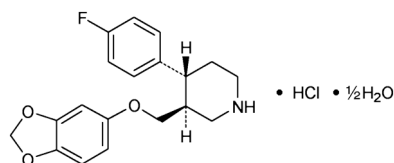
$C_{19}H_{20}FNO_3.HCl.\frac{1}{2}H_2O$: 374.83
(3S,4R)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine monohydrochloride hemihydrate
[*110429-35-1*]

Paroxetine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.5% of paroxetine hydrochloride ($C_{19}H_{20}FNO_3.HCl$: 365.83), calculated on the anhydrous basis.

Description Paroxetine Hydrochloride Hydrate occurs as a white crystalline powder.

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

Optical rotation $[\alpha]_D^{20}$: −83 – −93°(0.1 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Melting point: about 140°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Paroxetine Hydrochloride Hydrate in ethanol (99.5) (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 Paroxetine 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 Paroxetine Hydrochloride Hydrate 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 Paroxetine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Paroxetine Hydrochloride Hydrate (1 in 500) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Paroxetine Hydrochloride Hydrate according to Method 4, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 30). Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) 4-(4-Fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine—Dissolve 0.42 g of Paroxetine Hydrochloride Hydrate

in 10 mL of a mixture of water and acetonitrile (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (4:1) to make exactly 100 mL. Pipet 1 mL of this solution, and add a mixture of water and acetonitrile (4:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of water and acetonitrile (4:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 75 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 0.8 to paroxetine, obtained from the sample solution is not larger than the peak area of paroxetine obtained from the standard solution. For the area of the peak, having the relative retention time of about 0.8 to paroxetine, multiply the relative response factor 0.86.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 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 30°C.

Mobile phase A: Dissolve 30 g of sodium perchlorate monohydrate in 900 mL of water, add 3.5 mL of phosphoric acid, 2.4 mL of triethylamine and water to make 1000 mL, and then adjust to pH 2.0 with phosphoric acid or 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 – 20	85 → 80	15 → 20
20 – 27	80 → 55	20 → 45
27 – 36	55	45

Flow rate: 1.5 mL per minute.

System suitability—

System performance: When the procedure is run with 75 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 75 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 5.0%.

(3) Related substances—Dissolve 20 mg of Paroxetine Hydrochloride Hydrate in 20 mL of a mixture of water and tetrahydrofuran (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and tetrahydrofuran (9:1) to make exactly 100 mL. Pipet 1 mL of this solution, and add a mixture of water and tetrahydrofuran (9:1) 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 by the automatic integration method: the area of the peak other than paroxetine obtained from the sample solu-

tion is not larger than the peak area of paroxetine obtained from the standard solution. For the areas of the peaks, having the relative retention time of about 0.29, about 0.66, about 0.73, about 0.85, about 0.91, about 1.14, about 1.51, and about 1.84 to paroxetine, multiply their relative response factors 0.46, 0.82, 1.10, 0.95, 0.93, 0.82, 1.55, and 1.54, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 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 A: A mixture of water, tetrahydrofuran and trifluoroacetic acid (180:20:1).

Mobile phase B: A mixture of acetonitrile, tetrahyrofuran and trifluoroacetic acid (180:20:1).

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 – 30	80	20
30 – 50	80 → 20	20 → 80
50 – 60	20	80

Flow rate: 1.0 mL per minute.

Time span of measurement: For 60 minutes after injection, 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 paroxetine 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 paroxetine is not more than 2.0%.

(4) Optical isomer—Dissolve 0.10 g of Paroxetine Hydrochloride Hydrate in 20 mL of methanol, add a solution of sodium chloride (29 in 1000) to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 10 mL of methanol, and add a solution of sodium chloride (29 in 1000) to make exactly 50 mL. Pipet 2 mL of this solution, add 4 mL of methanol, and add a solution of sodium chloride (29 in 1000) 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 of the optical isomer, having the relative retention time of about 0.4 to paroxetine, obtained from the sample solution is not larger than the peak area of paroxetine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4 mm in inside diameter and 10 cm in length, packed with α_1-acid glycoprotein-binding silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 18°C.

Mobile phase: A mixture of sodium chloride solution (29 in 1000) and methanol (4:1).

Flow rate: Adjust so that the retention time of paroxetine is about 22 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 paroxetine are not less than 500 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 paroxetine is not more than 2.0%.

Water <2.48> 2.0 – 3.0% (0.2 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 50 mg each of Paroxetine Hydrochloride Hydrate and Paroxetine Hydrochloride RS (separately determine the water <2.48> in the same manner as Paroxetine Hydrochloride Hydrate), dissolve them separately 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 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 paroxetine in each solution.

$$\begin{aligned} &\text{Amount (mg) of paroxetine hydrochloride} \\ &(C_{19}H_{20}FNO_3 \cdot HCl) \\ &\quad = M_S \times A_T/A_S \end{aligned}$$

M_S: Amount (mg) of Paroxetine Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

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

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

Mobile phase: Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and adjust to pH 4.5 with acetic acid (100). To 600 mL of this solution, add 400 mL of acetonitrile and 10 mL of triethylamine, then adjust to pH5.5 with acetic acid (100).

Flow rate: Adjust so that the retention time of paroxetine 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 paroxetine 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 paroxetine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Paroxetine Hydrochloride Tablets

パロキセチン塩酸塩錠

Paroxetine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$: 329.37).

Method of preparation Prepare as directed under Tablets, with Paroxetine Hydrochloride Hydrate.

Identification Powder Paroxetine Hydrochloride Tablets. To a portion of the powder, equivalent to 10 mg of paroxetine ($C_{19}H_{20}FNO_3$), add 140 mL of ethanol (99.5), treat with the aid of ultrasonic waves for 5 minutes, add ethanol (99.5) to make 200 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 233 nm and 237 nm, between 263 nm and 267 nm, between 269 nm and 273 nm, and between 293 nm and 297 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 Paroxetine Hydrochloride Tablets add $V/5$ mL of 0.1 mol/L hydrochloric acid TS, disintegrate with the aid of ultrasonic waves for 10 minutes, add $3V/5$ mL of a mixture of water and 2-propanol (1:1), and treat with the ultrasonic waves for 20 minutes. To this solution add a mixture of water and 2-propanol (1:1) to make exactly V mL so that each mL contains about 0.2 mg of paroxetine ($C_{19}H_{20}FNO_3$), 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.

$$\begin{aligned} &\text{Amount (mg) of paroxetine } (C_{19}H_{20}FNO_3) \\ &\quad = M_S \times A_T/A_S \times V/100 \times 0.900 \end{aligned}$$

M_S: Amount (mg) of Paroxetine 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 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of 5-mg and 10-mg tablet is not less than 80%, and of 20-mg tablet is not less than 75%.

Start the test with 1 tablet of Paroxetine 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 5.6 μg of paroxetine ($C_{19}H_{20}FNO_3$), and use this solution as the sample solution. Separately, weigh accurately about 11 mg of Paroxetine Hydrochloride RS (separately determine the water <2.48> in the same manner as Paroxetine Hydrochloride Hydrate), and dissolve in the dissolution medium to make exactly 100 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 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 paroxetine in each solution.

Dissolution rate (%) with respect to the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 54 \times 0.900$$

M_S: Amount (mg) of Paroxetine Hydrochloride RS taken, calculated on the anhydrous basis

C: Labeled amount (mg) of paroxetine ($C_{19}H_{20}FNO_3$) 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 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 2.0%.

Assay   Weigh accurately the mass of not less than 20 Paroxetine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of paroxetine ($C_{19}H_{20}FNO_3$), add 20 mL of 0.1 mol/L hydrochloric acid TS, treat with the aid of ultrasonic waves for 10 minutes. To this solution add 60 mL of a mixture of water and 2-propanol (1:1), and treat with the aid of ultrasonic waves for 20 minutes. Then add a mixture of water and 2-propanol (1:1) to make exactly 100 mL, 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 23 mg of Paroxetine Hydrochloride RS (separately determine the water <2.48> in the same manner as Paroxetine Hydrochloride Hydrate), and dissolve in 20 mL of 0.1 mol/L hydrochloric acid TS, add a mixture of water and 2-propanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 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 paroxetine in each solution.

$$\text{Amount (mg) of paroxetine } (C_{19}H_{20}FNO_3)$$
$$= M_S \times A_T/A_S \times 0.900$$

M_S: Amount (mg) of Paroxetine Hydrochloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

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

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

Mobile phase: Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and adjust to pH 4.5 with acetic acid (100). To 600 mL of this solution, add 400 mL of acetonitrile and 10 mL of triethylamine, then adjust to pH 5.5 with acetic acid (100).

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

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry

factor of the peak of paroxetine are not less than 5000 and not more than 3.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Pemirolast Potassium

ペミロラストカリウム
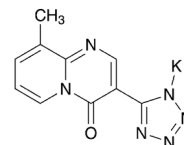
$C_{10}H_7KN_6O$: 266.30
Monopotassium 5-(9-methyl-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)-1H-tetrazol-1-ide
[*100299-08-9*]

Pemirolast Potassium contains not less than 98.5% and not more than 101.0% of pemirolast potassium ($C_{10}H_7KN_6O$), calculated on the anhydrous basis.

Description   Pemirolast Potassium occurs as a light yellow crystalline powder.

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

It dissolves in potassium hydroxide TS.

Melting point: about 322°C (with decomposition).

Identification   **(1)**   Determine the absorption spectrum of a solution of Pemirolast Potassium in diluted potassium hydroxide TS (1 in 10,000) (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 Pemirolast Potassium 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 Pemirolast Potassium 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 Pemirolast Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)   Pemirolast Potassium responds to the Qualitative Tests <1.09> (1) for potassium salt.

Purity   **(1)**   Clarity of solution—A solution obtained by dissolving 0.5 g of Pemirolast Potassium in 10 mL of water is clear.

(2)   Heavy metals <1.07>—Proceed with 0.5 g of Pemirolast Potassium 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).

(3)   Related substances—Dissolve 50 mg of Pemirolast Potassium in 50 mL of a mixture of phosphate buffer solution (pH 8.0) and methanol (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of phosphate buffer solution (pH 8.0) and methanol (3:2) to make exactly 100 mL. To exactly 2.5 mL of this solution add a mixture of phosphate buffer solution (pH 8.0) and methanol (3:2) to make exactly 50 mL, and use this solu-

tion 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 pemirolast obtained from the sample solution is not larger than the peak area of pemirolast 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 9 times as long as the retention time of pemirolast.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of phosphate buffer solution (pH 8.0) and methanol (3:2) to make exactly 25 mL. Confirm that the peak area of pemirolast 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 pemirolast are not less than 3000 and not more than 1.7, 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 pemirolast is not more than 2.0%.

Water <2.48> Not more than 0.5% (0.1 g, coulometric titration).

Assay Weigh accurately about 50 mg each of Pemirolast Potassium and Pemirolast Potassium RS (separately determine the water <2.48> in the same manner as Pemirolast Potassium), dissolve in a mixture of phosphate buffer solution (pH 8.0) and methanol (3:2) to make them exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add a mixture of phosphate buffer solution (pH 8.0) and methanol (3:2) 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 area of pemirolast to that of the internal standard.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Pemirolast Potassium RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 1000).

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 25°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (30:20:1).

Flow rate: Adjust so that the retention time of pemirolast

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, pemirolast 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 pemirolast to that of the internal standard is not more than 1.0%.

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

Pemirolast Potassium Ophthalmic Solution

ペミロラストカリウム点眼液

Pemirolast Potassium Ophthalmic Solution is an aqueous ophthalmic preparation.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$: 266.30).

Method of preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Pemirolast Potassium.

Description Pemirolast Potassium Ophthalmic Solution is a clear, colorless liquid.

Identification To a volume of Pemirolast Potassium Ophthalmic Solution, equivalent to 1 mg of Pemirolast Potassium, add diluted 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 10) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 255 nm and 259 nm, and between 355 nm and 359 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 a volume of Pemirolast Potassium Ophthalmic Solution, equivalent to 2 mg of Pemirolast Potassium, add 1 mL of methanol and diluted 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 10) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 20 mL of methanol and diluted 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 10) 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 pemirolast obtained from the sample solution is not larger than 3/10 times the peak area of pemirolast obtained from the standard solution, and the total area of the peaks other than pemirolast from the sample solution is not larger than the peak area of pemirolast from the standard solution.

Operating conditions—

Detector: An ultraviolet spectrophotometer (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: A mixture of trifluoroacetic acid TS and methanol (4:1).

Mobile phase B: A mixture of methanol and trifluoroacetic acid TS (3:2).

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 – 60	100 → 0	0 → 100

Flow rate: Adjust so that the retention time of pemirolast is about 19 minute.

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

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add diluted 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 10) to make exactly 20 mL. Confirm that the peak area of pemirolast 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: Dissolve 10 mg of pemirolast potassium in 10 mL of diluted 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 10), transfer this solution to a colorless test tube, and illuminate with a D_{65} fluorescent lamp (3000 lx) for 72 hours. To 2 mL of this solution add 1 mL of methanol and diluted 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 10) to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak, having the relative retention time about 0.9 to pemirolast, and the peak of pemirolast is 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 pemirolast is not more than 2.0%.

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 Pipet a volume of Pemirolast Potassium Ophthalmic Solution, equivalent to 2 mg of pemirolast potassium ($C_{10}H_7KN_6O$), add exactly 2 mL of the internal standard solution, then add a mixture of diluted 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) (1 in 10) and methanol (3:2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Pemirolast Potassium RS (separately determine the water *<2.48>* in the same manner as Pemirolast Potassium), and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add a mixture of diluted 0.1 mol/L phosphate buffer

solution for antibiotics (pH 8.0) (1 in 10) and methanol (3:2) 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. Calculate the ratios, Q_T and Q_S of the peak area of pemirolast to that of the internal standard.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
$$= M_S \times Q_T/Q_S \times 1/25$$

M_S: Amount (mg) of Pemirolast Potassium RS taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of ethyl aminobenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 260 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 40°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (30:20:1).

Flow rate: Adjust so that the retention time of pemirolast is about 4 minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pemirolast 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 pemirolast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pemirolast Potassium for Syrup

シロップ用ペミロラストカリウム

Pemirolast Potassium for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$: 266.30).

Method of preparation Prepare as directed under Preparations for Syrups, with Pemirolast Potassium.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits maxima between 255 nm and 259 nm and between 355 nm and 359 nm.

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

Uniformity of dosage units *<6.02>* Perform the test according to the following method: Pemirolast Potassium for Syrup in single-dose packages meet the requirement of the Content uniformity test.

Dissolve the total amount of the content of 1 package of

Pemirolast Potassium for Syrup in water to make exactly V mL so that each mL contains about 50 μg of pemirolast potassium ($C_{10}H_7KN_6O$). Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
  $= M_S \times A_T/A_S \times V/400$

M_S: Amount (mg) of Pemirolast Potassium RS taken, calculated on the anhydrous basis

Assay Powder Pemirolast Potassium for Syrup. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium ($C_{10}H_7KN_6O$), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS (separately determine the water ⟨2.48⟩ in the same manner as Pemirolast Potassium), 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, at 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
  $= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of Pemirolast Potassium RS taken, calculated on the anhydrous basis

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

Pemirolast Potassium Tablets

ペミロラストカリウム錠

Pemirolast Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$: 266.30).

Method of preparation Prepare as directed under Tablets, with Pemirolast Potassium.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩: it exhibits maxima between 255 nm and 259 nm, and between 355 nm and 359 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 Pemirolast Potassium Tablets add 50 mL of water for 5 mg of pemirolast potassium ($C_{10}H_7KN_6O$), and shake to disintegrate the tablet completely. Then, add water to make exactly V mL so that each mL contains about 50 μg of pemirolast potassium ($C_{10}H_7KN_6O$), and filter. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
  $= M_S \times A_T/A_S \times V/400$

M_S: Amount (mg) of Pemirolast Potassium 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 disodium hydrogen phosphate-citric acid buffer solution (pH 5.0) as the dissolution medium, the dissolution rate in 45 minutes of a 5-mg tablet is not less than 75%, and that in 60 minutes of a 10-mg tablet is not less than 70%.

Start the test with 1 tablet of Pemirolast Potassium 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 5.6 μg of pemirolast potassium ($C_{10}H_7KN_6O$). Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Pemirolast Potassium RS (separately determine the water ⟨2.48⟩ in the same manner as Pemirolast Potassium), dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL. Pipet 4 mL of this solution, add exactly 2 mL of diluted potassium hydroxide TS (1 in 10), and use this solution as the standard solution. Then, proceed as directed in the Assay.

Dissolution rate (%) with respect to the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$)
  $= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Pemirolast Potassium RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$) in 1 tablet

Assay Accurately weigh the mass of not less than 20 Pemirolast Potassium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of pemirolast potassium ($C_{10}H_7KN_6O$), add 50 mL of water, shake thoroughly for 20 minutes, then add water to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pemirolast Potassium RS (separately determine the water ⟨2.48⟩ in the same manner as Pemirolast Potassium), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 1 mL of diluted potassium hydroxide TS (1 in 100), add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T sand A_S, at 357 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, using water as the blank.
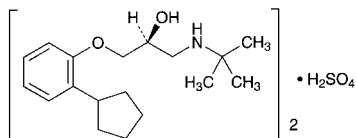
Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
  $= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of Pemirolast Potassium RS taken, calculated on the anhydrous basis

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

Penbutolol Sulfate

ペンブトロール硫酸塩

$(C_{18}H_{29}NO_2)_2.H_2SO_4$: 680.94
(2*S*)-3-(2-Cyclopentylphenoxy)-1-
(1,1-dimethylethyl)aminopropan-2-ol hemisulfate
[*38363-32-5*]

Penbutolol Sulfate, when dried, contains not less than 98.5% of penbutolol sulfate [$(C_{18}H_{29}NO_2)_2.H_2SO_4$].

Description   Penbutolol Sulfate occurs as a white crystalline powder.

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

Identification   (1)   Determine the absorption spectrum of a solution of Penbutolol Sulfate 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 Penbutolol Sulfate, previously dried, 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 0.1 g of Penbutolol Sulfate in 25 mL of water by warming, and cool: this solution responds to Qualitative Tests <*1.09*> for sulfate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: $-23 - -25°$ (after drying, 0.2 g, methanol, 20 mL, 100 mm).

Melting point <*2.60*>   213 – 217°C

Purity   (1)   Heavy metals <*1.07*>—Proceed with 2.0 g of Penbutolol Sulfate 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 Penbutolol Sulfate according to Method 4, and perform the test (not more than 2 ppm).

(3)   Related substances—Dissolve 0.8 g of Penbutolol Sulfate 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 2-propanol, ethanol (95) and ammonia solution (28) (85:12:3) 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% (0.5 g, 105°C, 3 hours).

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

Assay   Weigh accurately about 0.8 g of Penbutolol Sulfate, 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
= 68.09 mg of $(C_{18}H_{29}NO_2)_2.H_2SO_4$

Containers and storage   Containers—Well-closed containers.

Pentazocine

ペンタゾシン

and enantiomer
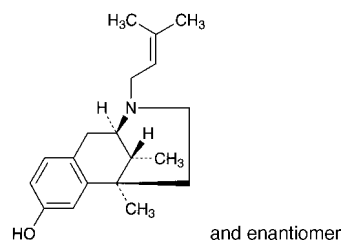
$C_{19}H_{27}NO$: 285.42
(2*RS*,6*RS*,11*RS*)-6,11-Dimethyl-
3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-
2,6-methano-3-benzoazocin-8-ol
[*359-83-1*]

Pentazocine, when dried, contains not less than 99.0% of pentazocine ($C_{19}H_{27}NO$).

Description   Pentazocine occurs as a white to pale yellowish white, crystalline powder. It is odorless.

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

Identification   (1)   To 1 mg of Pentazocine add 0.5 mL of formaldehyde-sulfuric acid TS: a deep red color is produced, and it changes to grayish brown immediately.

(2)   Dissolve 5 mg of Pentazocine in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and heat in a water bath for 2 minutes: the color of the solution changes from light yellow to deep yellow. Shake the solution with 1 drop of nitric acid: the solution remains yellow in color.

(3)   Determine the absorption spectrum of a solution of Pentazocine in 0.01 mol/L hydrochloric acid TS (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.

Absorbance <*2.24*> $E_{1\,cm}^{1\%}$ (278 nm): 67.5 – 71.5 (after drying, 0.1 g, 0.01 mol/L hydrochloric acid TS, 1000 mL).

Melting point <*2.60*>   150 – 158°C

Purity   (1)   Clarity and color of solution—Dissolve 0.10 g of Pentazocine in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2)   Heavy metals <*1.07*>—Proceed with 1.0 g of Pentazocine 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 Pentazocine according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentazocine 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 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, methanol and isopropylamine (94:3:3) to a distance of about 13 cm, and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: any 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, 60°C, 5 hours).

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

Assay   Weigh accurately about 0.5 g of Pentazocine, 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.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 28.54 \text{ mg of } C_{19}H_{27}NO$$

Containers and storage   Containers—Well-closed containers.

Pentobarbital Calcium

ペントバルビタールカルシウム
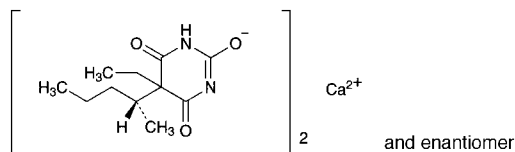
$C_{22}H_{34}CaN_4O_6$: 490.61
Monocalcium bis[5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidin-2-olate]
[*76-74-4*, Pentobarbital]

Pentobarbital Calcium contains not less than 98.0% and not more than 102.0% of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$), calculated on the dried basis.

Description   Pentobarbital Calcium occurs as a white powder.

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

A solution of Pentobarbital Calcium (1 in 100) shows no optical rotation.

Identification   (1)   Determine the infrared absorption spectrum of Pentobarbital Calcium 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)   To 1 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, shake with 5 mL of dilute hydrochloric acid and 10 mL of water, allow to cool, and filter. To the filtrate add 1 drop of methyl red TS, and add ammonia TS until a slight yellow color develops: the solution responds to Qualitative Tests <*1.09*> (1), (2) and (3) for calcium salt.

Purity   (1)   Chloride <*1.03*>—To 1.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 2.5 mL of dilute nitric acid, dissolve by warming with shaking, cool, add water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, and to 15 mL of the subsequent 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 as follows: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 1.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2)   Heavy metals <*1.07*>—To 2.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, cool, add water to make 80 mL, shake well, and filter. Discard the first 10 mL of the filtrate, to 40 mL of the subsequent filtrate add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, 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 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 30 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, then add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3)   Related substances—Dissolve 10 mg of Pentobarbital Calcium in 100 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 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 areas of each peak by the automatic integration method: the area of any peak other than the peak of pentobarbital from the sample solution is not larger than 3/10 times the peak area of pentobarbital from the standard solution, and the total of these peak area is not larger than the peak area of pentobarbital 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 pentobarbital, beginning after the solvent peak.

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

Test for required detectability: Pipet 2 mL of the standard solution, add water to make exactly 20 mL, and confirm that the peak area of pentobarbital obtained from 20 μL of this solution is equivalent to 5 to 15% of that obtained from 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 areas of pentobarbital is not more than 5%.

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 5 hours).

Assay Weigh accurately about 20 mg of Pentobarbital Calcium, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Pentobarbital RS, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water 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 pentobarbital to that of the internal standard.

Amount (mg) of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$)
$= M_S \times Q_T/Q_S \times 1.084$

M_S: Amount (mg) of Pentobarbital RS taken

Internal standard solution—Dissolve 0.2 g of isopropyl parahydroxybenzoate in 20 mL of acetonitorile, and add water to make 100 mL.
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 40°C.
Mobile phase: Dissolve 1.36 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitorile.
Flow rate: Adjust so that the retention time of pentobarbital 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, pentobarbital 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pentobarbital to that of the internal standard is not more than 1.0%.
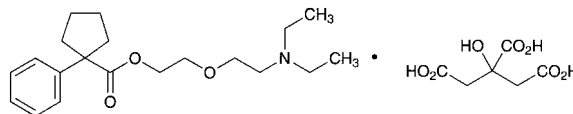
Containers and storage Containers—Well-closed containers.

Pentoxyverine Citrate

Carbetapentane Citrate
Carbetapentene Citrate

ペントキシベリンクエン酸塩

$C_{20}H_{31}NO_3.C_6H_8O_7$: 525.59
2-[2-(Diethylamino)ethoxy]ethyl
1-phenylcyclopentanecarboxylate monocitrate
[23142-01-0]

Pentoxyverine Citrate, when dried, contains not less than 98.5% of pentoxyverine citrate ($C_{20}H_{31}NO_3$. $C_6H_8O_7$).

Description Pentoxyverine Citrate occurs as a white, crystalline powder.
It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.1 g of Pentoxyverine Citrate in 10 mL of water, and add 10 mL of Reinecke salt TS: a light red precipitate is formed.
(2) Determine the infrared absorption spectrum of Pentoxyverine Citrate, previously dried, 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) A solution of Pentoxyverine Citrate (1 in 10) responds to Qualitative Tests <1.09> (1) and (2) for citrate.

Melting point <2.60> 92 – 95°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pentoxyverine Citrate in 10 mL of water: the solution is clear and colorless.
(2) Heavy metals <1.07>—Proceed with 2.0 g of Pentoxyverine Citrate 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 Pentoxyverine Citrate according to Method 3, and perform the test (not more than 2 ppm).
(4) Related substances—Dissolve 0.20 g of Pentoxyverine Citrate in 10 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 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 15 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of chloroform, methanol, ethyl acetate and ammonia solution (28) (25:10:10:1) to a distance of about 10 cm, and air-dry the plate. Allow to stand in 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 vacu-

um, phosphorus (V) oxide, 60°C, 4 hours).

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

Assay Weigh accurately about 0.5 g of Pentoxyverine Citrate, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L of perchloric acid VS until the color of the solution changes from purple through blue-green 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
= 52.56 mg of $C_{20}H_{31}NO_3 \cdot C_6H_8O_7$

Containers and storage Containers—Well-closed containers.

Peplomycin Sulfate

ペプロマイシン硫酸塩
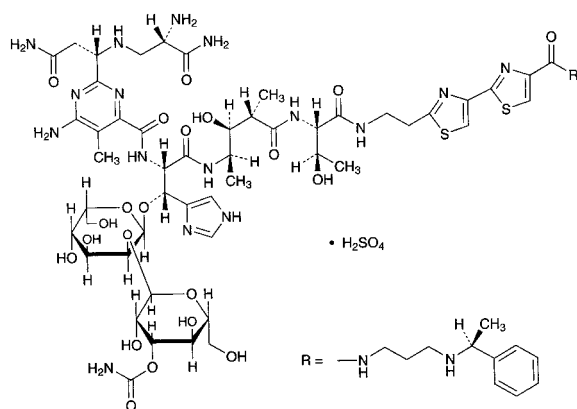
$C_{61}H_{88}N_{18}O_{21}S_2 \cdot H_2SO_4$: 1571.67
N^1-{3-[(1S)-(1-Phenylethyl)amino]propyl}bleomycinamide monosulfate
[*70384-29-1*]

Peplomycin Sulfate is the sulfate of a substance having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 865 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Peplomycin Sulfate is expressed as mass (potency) of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

Description Peplomycin Sulfate occurs as a white to light yellowish white powder.

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

It is hygroscopic.

Identification (1) To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water 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 or the spectrum of a solution of Peplomycin Sulfate 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 Peplomycin Sulfate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Peplomycin Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate RS in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), 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: the retention time of the principal peak in the chromatogram obtained from the sample solution is the same as that in the chromatogram obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(4) A solution of Peplomycin Sulfate (1 in 200) responds to the Qualitative Tests <1.09> (1) and (2) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: −2 – −5° (0.1 g calculated on the dried basis, 0.1 mol/L phosphate buffer solution (pH 5.3), 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Peplomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 80 mg of Peplomycin Sulfate in 4 mL of water: the solution is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Peplomycin Sulfate in exacty 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to 5.0 mL of Standard Copper Stock Solution add diluted nitric acid (1 in 100) to make exactly 100 mL. To 3.0 mL of this solution add diluted nitric acid (1 in 100) 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 Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene.
    Supporting gas—Air.
Lamp: Copper hollow cathode lamp.
Wavelength: 324.8 nm.

(3) Related substances—Dissolve about 10 mg of Peplomycin Sulfate in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), 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 areas of the peaks, appeared after the peak of copper sulfate, by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than peplomycin is not more than 7.0%.

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 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of mobile phase stock solution and methanol (3:2).

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 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: 1.2 mL per minute.

Time span of measurement: As long as 20 minutes after elution of peplomycin, beginning after the peak of copper sulfate.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add water 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 water to make exactly 10 mL. Confirm that the peak area of peplomycin 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 performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of peplomycin are not less than 30,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of peplomycin is not more than 2.0%.

Loss on drying *<2.41>*   Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Handle the sample avoiding absorption of moisture.

Assay   Weigh accurately an amount of Peplomycin Sulfate and Peplomycin Sulfate RS, both previously dried, equivalent to about 50 mg (potency), dissolve them separately in the mobile phase to make exactly 100 mL. Pipet 4 mL each of these solutions, add exactly 10 mL 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 1 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 peplomycin to that of the internal standard.

$$\text{Amount } [\mu g \text{ (potency)}] \text{ of peplomycin sulfate}$$
$$(C_{61}H_{88}N_{18}O_{21}S_2 \cdot H_2SO_4)$$
$$= M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Peplomycin Sulfate RS taken

Internal standard solution—A solution of 1-aminonaphthalene in mobile phase (1 in 20,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (2.2 μm in particle diameter).

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

Mobile phase: Dissolve 0.96 g of sodium 1-pentane sulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water, add 5 mL of acetic acid (100), and adjust to pH 4.3 with ammonia TS. To 650 mL of this solution add 350 mL of methanol.

Flow rate: Adjust so that the retention time of peplomycin is about 3 minutes.
System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, peplomycin 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 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of peplomycin to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Peplomycin Sulfate for Injection

注射用ペプロマイシン硫酸塩

Peplomycin Sulfate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled potency of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

Method of preparation   Prepare as directed under Injections, with Peplomycin Sulfate.

Description   Peplomycin Sulfate for Injection occurs as white light masses or powder.

Identification   Take an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate, and dissolve in 15 μL of Copper (II) sulfate TS and water to make 2 mL. Apply this solution to the column (prepared by filling a 15 mm inside diameter and 15 cm long chromatography tube with 15 mL of strongly basic ion exchange resin (Cl type) for column chromatography (75 – 150 μm in particle diameter) and run off. Then wash the column using water at 2.5 mL per minute, collect about 30 mL of the effluent. Add water to the effluent to make 250 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits maxima between 242 nm and 246 nm, and between 291 nm and 295 nm. Further determine the absorbances A_1 and A_2, at 243 nm and 293 nm, respectively: the ratio A_1/A_2 is 1.20 to 1.30.

Osmotic pressure ratio   Being specified separately when the drug is granted approval based on the Law.

pH *<2.54>*   The pH of a solution prepared by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 50 mg (potency) of Peplomycin Sulfate, in 10 mL of water is 4.5 to 6.0.

Purity   Clarity and color of solution—A solution prepared

by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate, in 10 mL of water is clear and colorless.

Loss on drying <2.41> Not more than 4.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Perform the sampling preventing from moisture absorption.

Bacterial endotoxins <4.01> Less than 1.5 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 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—*Mycobacterium smegmatis* ATCC 607

(ii) Agar media for base layer, seed and transferring test organisms—
Glycerin 10.0 g
Peptone 10.0 g
Meat extract 10.0 g
Sodium chloride 3.0 g
Agar 15.0 g
Water 1000 mL
Mix all the ingredients, and sterilize. Adjust to pH 6.9 to 7.1 with sodium hydroxide TS after sterilization.

(iii) Liquid media for suspending the test organism
Glycerin 10.0 g
Peptone 10.0 g
Meat extract 10.0 g
Sodium chloride 3.0 g
Water 1000 mL
Mix all the components, and sterilize. Adjust to pH 6.9 to 7.1 with sodium hydroxide TS after sterilization.

(iv) Preparation of seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(vi) Standard solutions—Weigh accurately an amount of Peplomycin Sulfate RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 6.8) 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 15 days. Measure exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make

solutions so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately the mass of the contents of not less than 10 containers of Peplomycin Sulfate for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Peplomycin Sulfate, dissolve in 0.1 mol/L phosphate buffer solution (pH 6.8) to make exactly 100 mL. Measure exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make solutions so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Perphenazine

ペルフェナジン
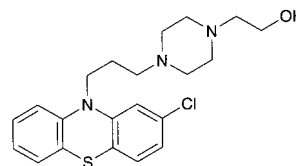
C$_{21}$H$_{26}$ClN$_3$OS: 403.97
2-{4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl}ethanol
[*58-39-9*]

Perphenazine, when dried, contains not less than 98.5% of perphenazine (C$_{21}$H$_{26}$ClN$_3$OS).

Description Perphenazine occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

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

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Identification (1) Dissolve 5 mg of Perphenazine in 5 mL of sulfuric acid: a red color, changing to deep red-purple upon warming, is produced.

(2) Dissolve 0.2 g of Perphenazine in 2 mL of methanol, add this solution to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and allow to stand for 4 hours. Collect the crystals, wash with a small volume of methanol, and dry at 105°C for 1 hour: the crystals so obtained melt <2.60> between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine 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 1 or the spectrum of a solution of Perphenazine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 10 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 2 or the spectrum of a solution of Perphena-

zine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4)   Perform the test with Perphenazine as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60>   95 – 100°C

Purity   (1)   Heavy metals <1.07>—Proceed with 1.0 g of Perphenazine 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 in the current of nitrogen in light-resistant containers under the protection from light. Dissolve 0.10 g of Perphenazine 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 10 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and 1 mol/L ammonia TS (5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the sample solution is not more intense than that from the standard solution.

Loss on drying <2.41>   Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 65°C, 4 hours).

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

Assay   Weigh accurately about 0.4 g of Perphenazine, 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 purple through blue-purple to blue-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
= 20.20 mg of $C_{21}H_{26}ClN_3OS$

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

Perphenazine Tablets

ペルフェナジン錠

Perphenazine Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$: 403.97).

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

Identification   (1)   Shake well a quantity of powdered Perphenazine Tablets, equivalent to 25 mg of Perphenazine, with 10 mL of methanol, and filter. Evaporate 2 mL of the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification (1) under Perphenazine.

(2)   Add 5 mL of the filtrate obtained in (1) to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and proceed as directed in the Identification (2) under Perphenazine.

(3)   Determine the absorption spectrum of the filtrate

obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 309 nm and 313 nm. Add 30 mL of methanol to another 10 mL of the filtrate, and determine the absorption spectrum: it exhibits a maximum between 256 nm and 260 nm.

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

Disintegrate 1 tablet of Perphenazine Tablets by shaking with 5 mL of water, shake well with 70 mL of methanol, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL of a solution containing about 4 μg of perphenazine ($C_{21}H_{26}ClN_3OS$) in each ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 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 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of perphenazine ($C_{21}H_{26}ClN_3OS$)
= $M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of Perphenazine RS taken

Dissolution <6.10>   When the test is performed at 100 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 90 minutes of Perphenazine Tablets is not less than 70%.

Start the test with 1 tablet of Perphenazine 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 10 mg of Perphenazine RS, previously dried in vacuum with phosphorus (V) oxide at 65°C for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 250 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 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$)
= $M_S \times A_T/A_S \times 1/C \times 18$

M_S: Amount (mg) of Perphenazine RS taken
C: Labeled amount (mg) of perphenazine ($C_{21}H_{26}ClN_3OS$) in 1 tablet

Assay   Weigh accurately and powder not less than 20 Perphenazine Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of perphenazine ($C_{21}H_{26}ClN_3OS$), add 70 mL of methanol, shake well, and add methanol to make exactly 100 mL. Filter the solution, and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 10 mg of Perphenazine RS, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, and dissolve in methanol to make exactly 250 mL. Pipet 5 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 the standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of perphenazine } (C_{21}H_{26}ClN_3OS)$$
$$= M_S \times A_T/A_S \times 2/5$$

M_S: Amount (mg) of Perphenazine RS taken

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

Perphenazine Maleate

ペルフェナジンマレイン酸塩
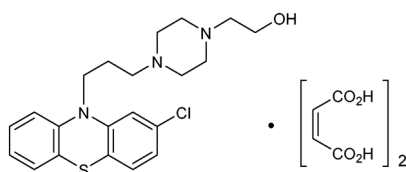
C$_{21}$H$_{26}$ClN$_3$OS.2C$_4$H$_4$O$_4$: 636.11
2-{4-[3-(2-Chloro-10H-phenothiazin-10-yl)propyl]piperazin-1-yl}ethanol dimaleate
[*58-39-9*, Perphenazine]

Perphenazine Maleate, when dried, contains not less than 98.0% of perphenazine maleate (C$_{21}$H$_{26}$ClN$_3$OS. 2C$_4$H$_4$O$_4$).

Description   Perphenazine Maleate occurs as a white to light yellow powder. It is odorless.

It is sparingly soluble in acetic acid (100), slightly soluble in water and in ethanol (95), and practically insoluble in chloroform.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 175°C (with decomposition).

Identification   (1)   Dissolve 8 mg of Perphenazine Maleate in 5 mL of sulfuric acid: a red color is produced, which becomes deep red-purple on warming.

(2)   Dissolve 0.3 g of Perphenazine Maleate in 3 mL of dilute hydrochloric acid, add 2 mL of water and 3 mL of ammonia solution (28), shake, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (5)]. Evaporate the combined chloroform extracts on a water bath to dryness, dissolve the residue in 20 mL of methanol, and pour into 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25). Allow to stand for 4 hours, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 237°C and 244°C (with decomposition).

(3)   Determine the absorption spectrum of a solution of Perphenazine Maleate (1 in 20,000) 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, to 10 mL of the solution add 30 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 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4)   Perform the test with Perphenazine Maleate as di-

rected under Flame Coloration Test <1.04> (2): a green color appears.

(5)   Evaporate the aqueous layer reserved in (2) to dryness. To the residue add 1 mL of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate in a water bath at about 35°C with the aid of a current of air: the residue melts <2.60> between 128°C and 136°C.

Purity   (1)   Heavy metals <1.07>—Proceed with 2.0 g of perphenazine 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)   Arsenic <1.11>—Prepare the test solution with 1.0 g of Perphenazine Maleate according to Method 3, and perform the test (not more than 2 ppm).

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 Perphenazine Maleate, previously dried, 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 blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 31.81 \text{ mg of } C_{21}H_{26}ClN_3OS.2C_4H_4O_4$$

Containers and storage   Containers—Well-closed containers.
Storage—Light-resistant.

Perphenazine Maleate Tablets

ペルフェナジンマレイン酸塩錠

Perphenazine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of perphenazine maleate (C$_{21}$H$_{26}$ClN$_3$OS. 2C$_4$H$_4$O$_4$: 636.11).

Method of preparation   Prepare as directed under Tablets, with Perphenazine Maleate.

Identification   (1)   Shake a quantity of powdered Perphenazine Maleate Tablets, equivalent to 0.04 g of Perphenazine Maleate, with 3 mL of dilute hydrochloric acid and 30 mL of water, centrifuge. Filter the supernatant liquid, add 3 mL of ammonia solution (28) to the filtrate, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (4).] Wash the combined chloroform extracts with two 5-mL portions of water, and separate the chloroform layer. Evaporate 6 mL of the chloroform solution on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Perphenazine Maleate.

(2)   Evaporate 20 mL of the chloroform solution obtained in (1) on a water bath to dryness, dissolve the residue in 20 mL of methanol, and filter, if necessary. Warm the filtrate, add 5 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), allow to stand for 4 hours, and proceed as directed in the Identification (2) under Perphenazine Maleate.

(3)   To 2 mL of the filtrate obtained in the Assay add water to make 50 mL. Determine the absorption spectrum of

the solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: it exhibits maxima between 253 nm and 257 nm and between 303 nm and 313 nm.

(4) Filter, if necessary, the aqueous layer reserved in (1), evaporate the filtrate to make about 5 mL, add 2 mL of dilute sulfuric acid, and extract with two 10-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate on a water bath to dryness, dissolve the residue in 5 mL of sulfuric acid TS, and add 1 to 2 drops of potassium permanganate TS: the red color of potassium permanganate TS fades immediately.

Uniformity of dosage units $\langle 6.02 \rangle$ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 tablet of Perphenazine Maleate Tablets by shaking with 15 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously with 50 mL of methanol, add water to make exactly 100 mL, and centrifuge. Pipet V mL of the supernatant liquid, add water to make exactly V' mL of a solution containing about 6 μg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$) in each ml, and use this solution as the sample solution. Separately, weigh accurately 30 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 0.1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 3 mL of 0.1 mol/L hydrochloric acid TS, 10 mL of methanol and water to make exactly 250 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 255 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank.

$$\text{Amount (mg) of perphenazine maleate}$$
$$(C_{21}H_{26}ClN_3OS.2C_4H_4O_4)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/50$$

M_S: Amount (mg) of perphenazine maleate for assay taken

Dissolution $\langle 6.10 \rangle$ 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 30 minutes of Perphenazine Maleate Tablets is not less than 70%.

Conduct this procedure without exposure to light. Start the test with 1 tablet of Perphenazine 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 the dissolution medium to make exactly V' mL so that each mL contains about 3.5 μg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 200 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 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$.

Dissolution rate (%) with respect to the labeled amount of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/4$$

M_S: Amount (mg) of perphenazine maleate for assay

taken

C: Labeled amount (mg) of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$) in 1 tablet

Assay   Weigh accurately and powder not less than 20 Perphenazine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$), shake well with 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 5 mL of the subsequent filtrate, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 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 255 nm as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank.

$$\text{Amount (mg) of perphenazine maleate}$$
$$(C_{21}H_{26}ClN_3OS.2C_4H_4O_4)$$
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of perphenazine maleate for assay taken

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

Adsorbed Purified Pertussis Vaccine

沈降精製百日せきワクチン

Adsorbed Purified Pertussis Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing the protective antigen of *Bordetella pertussis* to make the antigen insoluble.
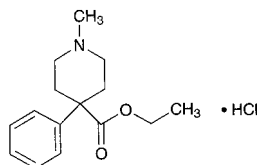
It conforms to the requirements of Adsorbed Purified Pertussis Vaccine in the Minimum Requirements for Biological Products.

Description   Adsorbed Purified Pertussis Vaccine forms a homogeneous, white turbidity on shaking.

Pethidine Hydrochloride

Operidine

ペチジン塩酸塩

$C_{15}H_{21}NO_2.HCl$: 283.79
Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate
monohydrochloride
[50-13-5]

Pethidine Hydrochloride, when dried, contains not less than 98.0% of pethidine hydrochloride ($C_{15}H_{21}NO_2.HCl$).

Description Pethidine Hydrochloride occurs as a white, crystalline powder.

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

The pH of a solution dissolved 1.0 g of Pethidine Hydrochloride in 20 mL of water is between 3.8 and 5.8.

Identification (1) Determine the absorption spectrum of a solution of Pethidine 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 Pethidine 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 Pethidine Hydrochloride (1 in 50) responds to Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 187 – 189°C

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

(2) Sulfate <1.14>—Perform the test with 0.20 g of Pethidine Hydrochloride. 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 0.05 g of Pethidine 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 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 both solutions by the automatic integration method: the total area of the peaks other than pethidine from the sample solution is not larger than the peak area of perthidine from the standard solution.
Operating conditions—
Detector: An ultraviolet absorption photometer (wave-length: 257 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 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

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

Time span of measurement: About 2 times as long as the retention time of pethidine, 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 20 mL. Confirm that the peak area of pethidine obtained from 20 μL of this solution is equivalent to 5 to 15% of that obtained from 20 μL of the standard solution.

System performance: To 2 mL each of the sample solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50,000) add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution according to the above operating conditions, pethidine and isoamyl 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 pethidine 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% (0.5 g).

Assay Weigh accurately about 0.5 g of Pethidine 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
= 28.38 mg of $C_{15}H_{21}NO_2.HCl$

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

Pethidine Hydrochloride Injection

Operidine Injection

ペチジン塩酸塩注射液

Pethidine Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pethidine hydrochloride ($C_{15}H_{21}NO_2.HCl$: 283.79).

Method of preparation Prepare as directed under Injections, with Pethidine Hydrochloride.

Description Pethidine Hydrochloride Injection is a clear, colorless liquid.

It is affected by light.
pH 4.0 – 6.0

Identification Take a volume of Pethidine Hydrochloride Injection equivalent to 0.1 g of Pethidine Hydrochloride, and add water to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Bacterial endotoxins <4.01> Less than 6.0 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 Measure exactly a volume of Pethidine Hydrochloride Injection, equivalent to about 0.1 g of pethidine hydrochloride ($C_{15}H_{21}NO_2.HCl$), add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this 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 pethidine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as 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, Q_T and Q_S, of the peak area of pethidine to that of the internal standard.

Amount (mg) of pethidine hydrochloride ($C_{15}H_{21}NO_2.HCl$)
  $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of pethidine hydrochloride for assay taken

Internal standard solution—A solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 12,500).
Operating conditions—
  Detector: An ultraviolet absorption photometer (wavelength: 257 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 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.
  Flow rate: Adjust so that the retention time of pethidine 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, pethidine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pethidine 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.

White Petrolatum

白色ワセリン

White Petrolatum is a decolorized and purified mixture of hydrocarbons obtained from petroleum.

Description White Petrolatum is a white to pale yellow, homogeneous, unctuous mass. It is odorless and tasteless.
  It is practically insoluble in water, in ethanol (95) and in ethanol (99.5).
  It dissolves in diethyl ether making a clear liquid or producing slight insoluble substances.
  It becomes a clear liquid when warmed.

Melting point <2.60> 38 – 60°C (Method 3).

Purity (1) Color—Melt White Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.
  Control solution: Add 3.4 mL of water to 1.6 mL of Iron (III) Chloride CS.
  (2) Acidity or alkalinity—To 35.0 g of White Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the White Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.
  (3) Heavy metals <1.07>—Proceed with 1.0 g of White Petrolatum 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).
  (4) Arsenic <1.11>—Prepare the test solution with 1.0 g of White Petrolatum, 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).
  (5) Sulfur compound—To 4.0 g of White Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.
  (6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of White Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.
  (7) Fats and fatty oils or resins—To 10.0 g of White Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

Residue on ignition <2.44> Not more than 0.05% (2 g).

Containers and storage Containers—Tight containers.

Hydrophilic Petrolatum

親水ワセリン

Method of preparation

White Beeswax	80 g
Stearyl Alcohol or Cetanol	30 g
Cholesterol	30 g
White Petrolatum	a sufficient quantity
	To make 1000 g

Melt and mix Stearyl Alcohol or Cetanol, White Beeswax and White Petrolatum on a water bath. Add Cholesterol, and melt completely by stirring. Stop warming, and stir until the mixture congeals.

Description Hydrophilic Petrolatum is white in color. It has a slight, characteristic odor.

When mixed with an equal volume of water, it retains the consistency of ointment.

Containers and storage Containers—Tight containers.

Yellow Petrolatum

黄色ワセリン

Yellow Petrolatum is a purified mixture of hydrocarbons obtained from petroleum.

Description Yellow Petrolatum occurs as a yellow, homogeneous, unctuous mass, It is odorless and tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in diethyl ether, in petroleum benzine and in turpentine oil, making a clear liquid or producing slight insoluble substances.

It becomes a yellow, clear liquid with slight fluorescence when warmed.

Melting point <2.60> 38 – 60°C (Method 3).

Purity (1) Color—Melt Yellow Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: To 3.8 mL of Iron (III) Chloride CS add 1.2 mL of Cobalt (II) Chloride CS.

(2) Acidity or alkalinity—To 35.0 g of Yellow Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the Yellow Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Yellow Petrolatum 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).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Yellow Petrolatum, 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).

(5) Sulfur compound—To 4.0 g of Yellow Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of Yellow Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of Yellow Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

Residue on ignition <2.44> Not more than 0.05% (2 g).

Containers and storage Containers—Tight containers.

Petroleum Benzin

石油ベンジン

Petroleum Benzin is a mixture of low-boiling point hydrocarbons from petroleum.

Description Petroleum Benzin occurs as a colorless, clear, volatile liquid. It shows no fluorescence. It has a chracteristic odor.

It is miscible with ethanol (99.5) and with diethyl ether.

It is practically insoluble in water.

It is very flammable.

Specific gravity d^{20}_{20}: 0.65 – 0.71

Purity (1) Acid—Shake vigorously 10 mL of Petroleum Benzin with 5 mL of water for 2 minutes, and allow to stand: the separated aqueous layer does not change moistened blue litmus paper to red.

(2) Sulfur compounds and reducing substances—To 10 mL of Petroleum Benzin add 2.5 mL of ammonia-ethanol TS and 2 to 3 drops of silver nitrate TS, and warm the mixture at about 50°C for 5 minutes, protected from light: no brown color develops.

(3) Fatty oil and sulfur compounds—Drop and evaporate 10 mL of Petroleum Benzin in small portions on odorless filter paper spread on a previously warmed glass plate: no spot or no foreign odor is perceptible.

(4) Benzene—Warm 5 drops of Petroleum Benzin with 2 mL of sulfuric acid and 0.5 mL of nitric acid for about 10 minutes, allow to stand for 30 minutes, transfer the mixture to a porcelain dish, and dilute with water: no odor of nitrobenzene is perceptible.

(5) Residue on evaporation—Evaporate 140 mL of Petroleum Benzin on a water bath to dryness, and heat the residue at 105°C to constant mass: the mass is not more than 1 mg.

(6) Readily carbonizable substances—Shake vigorously 5 mL of Petroleum Benzin with 5 mL of sulfuric acid for readily carbonizable substances for 5 minutes in a Nessler tube, and allow to stand: the sulfuric acid layer has no more color than Matching Fluid A.

Distilling range <2.57> 50 – 80°C, not less than 90 vol%.

Containers and storage   Containers—Tight containers.
Storage—Remote from fire, and not exceeding 30°C.

Phenethicillin Potassium

フェネチシリンカリウム

and epimer at C*
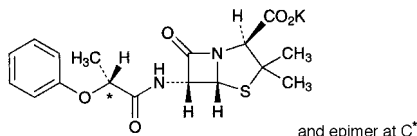
$C_{17}H_{19}KN_2O_5S$: 402.51
Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-
[(2*RS*)-2-phenoxypropanoylamino]-4-thia-1-
azabicyclo[3.2.0]heptane-2-carboxylate
[132-93-4]

Phenethicillin Potassium contains not less than 1400 units and not more than 1480 units per mg, calculated on the dried basis. The potency of Phenethicillin Potassium is expressed as unit based on the amount of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$). One unit of Phenethicillin Potassium is equivalent to 0.68 μg of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$).

Description   Phenethicillin Potassium occurs as a white to light yellowish white crystalline powder.

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

Identification   **(1)**   Determine the absorption spectrum of a solution of Phenethicillin Potassium (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 wavelengths.

(2)   Determine the infrared absorption spectrum of Phenethicillin Potassium 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)   Phenethicillin Potassium responds to Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +217 – +244° (1 g calculated on the dried basis, phosphate TS, 100 mL, 100 mm).

L-α-**Phenethicillin potassium**   Dissolve about 50 mg of Phenethicillin Potassium 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, and determine the peak areas, A_D and A_L, of D-α-phenethicillin and L-α-phenethicillin by the automatic integration method: $A_L/(A_D + A_L)$ is between 0.50 and 0.70.
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 30°C.

Mobile phase: Adjust the pH of a mixture of a solution of diammonium hydrogen phosphate (1 in 150) and acetonitrile

(41:10) to 7.0 with phosphoric acid.

Flow rate: Adjust so that the retention time of L-α-phenethicillin is about 25 minutes.
System suitability—

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, D-α-phenethicillin and L-α-phenethicillin 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 sample solution under the above operating conditions, the relative standard deviation of the peak area of L-α-phenethicillin is not more than 2.0%.

Purity   **(1)**   Heavy metals <1.07>—Proceed with 1.0 g of Phenethicillin Potassium 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 Phenethicillin Potassium according to Method 4 and, perform the test (not more than 2 ppm).

(3)   Related substances—Dissolve 50 mg of Phenethicillin Potassium in 50 mL of the mobile, 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 each peak area by the automatic integration method: the total of the peak areas other than D-α-phenethicillin and L-α-phenethicillin obtained from the sample solution is not larger than 5 times the total of the peak areas of D-α-phenethicillin and L-α-phenethicillin 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 L-α-Phenethicillin potassium.

Time span of measurement: About 1.5 times as long as the retention time of L-α-phenethicillin.
System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the L-α-Phenethicillin potassium.

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L-α-phenethicillin obtained from 10 μL of this solution is equivalent to 14 to 26% of that obtained from 10 μL of the standard solution.

Loss on drying <2.41>   Not more than 1.0% (0.1 g, in vacuum, 60°C, 3 hours).

Assay   Weigh accurately an amount of Phenethicillin Potassium and dried Phenethicillin Potassium RS, equivalent to about 40,000 units, dissolve each in phosphate buffer solution (pH 6.0) to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 2 mL each of these solutions in 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS to them, and allow to stand for exactly 15 minutes. To them add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, and allow them to stand for exactly 15 minutes. Add 0.2 – 0.5 mL of starch TS, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, to exactly 2 mL each of the sample solution and standard solution add exactly 10 mL of 0.005 mol/L iodine VS, then proceed in the

same manner as above without allowing to stand for 15 minutes as a blank determination, and make any necessary correction. Determine the volumes, V_T and V_S, of 0.005 mol/L iodine VS consumed in the sample solution and standard solution.
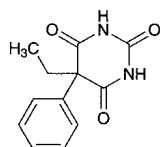
Amount (unit) of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$)
   = $M_S \times V_T/V_S$

M_S: Amount (unit) of Phenethicillin Potassium RS taken

Containers and storage   Containers—Well-closed containers.

Phenobarbital

フェノバルビタール

$C_{12}H_{12}N_2O_3$: 232.24
5-Ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione
[*50-06-6*]

Phenobarbital, when dried, contains not less than 99.0% and not more than 101.0% of phenobarbital ($C_{12}H_{12}N_2O_3$).

Description   Phenobarbital occurs as white crystals or crystalline powder.

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

It dissolves in sodium hydroxide TS.

The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

Identification   **(1)**   Determine the absorption spectrum of a solution of Phenobarbital in boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) (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 Phenobarbital 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>   175 – 179°C

Purity   **(1)**   Clarity and color of solution—Dissolve 0.5 g of Phenobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2)   Chloride <1.03>—Dissolve 0.30 g of Phenobarbital in 20 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: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3)   Heavy metals <1.07>—Proceed with 1.0 g of Phenobarbital 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)   Phenylbarbituric acid—Boil 1.0 g of Phenobarbital with 5 mL of ethanol (95) for 3 minutes: the solution is clear.

(5)   Related substances—Dissolve 0.10 g of Phenobarbital in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetonitrile to make exactly 100 mL. Pipet 5 mL of this 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, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than phenobarbital obtained from the sample solution is not larger than the peak area of phenobarbital 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 45°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

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

Time span of measurement: About 12 times as long as the retention time of phenobarbital, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of phenobarbital obtained with 10 μL of this solution is equivalent to 20 to 30% 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 phenobarbital are not less than 3000 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 phenobarbital is not more than 3.0%.

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 0.5 g of Phenobarbital, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution change from yellow to yellow-green (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination using a mixture of 50 mL of *N,N*-dimethylformamide and 22 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
   = 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and storage   Containers—Well-closed containers.

10% Phenobarbital Powder

Phenobarbital Powder

フェノバルビタール散 10%

10% Phenobarbital Powder contains not less than 9.3% and not more than 10.7% of phenobarbital ($C_{12}H_{12}N_2O_3$: 232.24).

Method of preparation

Phenobarbital	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 **(1)** 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 238 nm and 242 nm.

(2) To 6 g of 10% Phenobarbital Powder add 150 mL of ethanol, shake well, and filter. Condense the filtrate on a water bath to about 5 mL, add about 50 mL of water, filter to collect the formed crystals, and dry them at 105°C for 2 hours. Determine the infrared absorption spectrum of the crystals 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.

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 10% Phenobarbital Powder is not less than 80%.

Start the test with an accurately weighted about 0.3 g of 10% Phenobarbital Powder, 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 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) and use this solution as the sample solution. Separately, weigh accurately about 17 mg of phenobarbital 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, and add water to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) 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 a mixture of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) and water (2:1) as the blank, and determine the absorbances, A_T and A_S, at 240 nm.

Dissolution rate (%) with respect to the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$)

$$= M_S/M_T \times A_T/A_S \times 1/C \times 180$$

M_S: Amount (mg) of phenobarbital for assay taken
M_T: Amount (g) of 10% Phenobarbital Powder taken
C: Labeled amount (mg) of phenobarbital ($C_{12}H_{12}N_2O_3$) in 1 g

Assay Weigh accurately about 0.2 g of 10% Phenobarbital Powder, dissolve in a boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of phenobarbital for assay, previously dried at 105°C for 2 hours, and add a boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) 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 a boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.6) as the blank, and determine the absorbances, A_T and A_S, at 240 nm.

Amount (mg) of phenobarbital ($C_{12}H_{12}N_2O_3$)
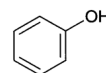$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of phenobarbital for assay taken

Containers and storage Containers—Well-closed containers.

Phenol

Carbolic Acid

フェノール

C_6H_6O: 94.11
Phenol
[*108-95-2*]

Phenol contains not less than 98.0% of phenol (C_6H_6O).

Description Phenol occurs as colorless to slightly red crystals or crystalline masses. It has a characteristic odor.

It is very soluble in ethanol (95) and in diethyl ether, and soluble in water.

Phenol (10 g) is liquefied by addition of 1 mL of water.

The color changes gradually through red to dark red by light or air.

It cauterizes the skin, turning it white.

Congealing point: about 40°C

Identification **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Purity **(1)** Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the

mass of the sample.

Assay   Dissolve about 1.5 g of Phenol, accurately weighed, in water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at once stopper the flask, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

<div align="center">

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

</div>

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

Liquefied Phenol

液状フェノール

Liquefied Phenol is Phenol maintained in a liquid condition by the presence of 10% of Water, Purified Water or Purified Water in Containers.

It contains not less than 88.0% of phenol (C_6H_6O: 94.11).

Description   Liquefied Phenol is a colorless or slightly reddish liquid. It has a characteristic odor.

It is miscible with ethanol (95), with diethyl ether and with glycerin.

A mixture of equal volumes of Liquefied Phenol and glycerin is miscible with water.

The color changes gradually to dark red on exposure to light or air.

It cauterizes the skin, turning it white.

Specific gravity d_{20}^{20}: about 1.065

Identification   (1)   Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Liquefied Phenol (1 in 100): a blue-purple color develops.

(2)   Add bromine TS dropwise to 5 mL of a solution of Liquefied Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Boiling point <*2.57*>   Not more than 182°C.

Purity   (1)   Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Liquefied Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2)   Residue on evaporation—Weigh accurately about 5 g of Liquefied Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

Assay   Dissolve about 1.7 g of Liquefied Phenol, accurately weighed, in a water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at one stopper the flask tightly, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate <*2.50*> the liber-

ated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

<div align="center">

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

</div>

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

Phenol for Disinfection

消毒用フェノール

Phenol for Disinfection contains not less than 95.0% of phenol (C_6H_6O: 94.11).

Description   Phenol for Disinfection occurs as colorless to slightly red crystals, crystalline masses, or liquid containing these crystals. It has a characteristic odor.

It is very soluble in ethanol (95) and in diethyl ether, and freely soluble in water.

Phenol for Disinfection (10 g) is liquefied by addition of 1 mL of water.

It cauterizes the skin, turning it white.

Congealing point: about 30°C.

Identification   (1)   To 10 mL of a solution of Phenol for Disinfection (1 in 100) add 1 drop of iron (III) chloride TS: a blue-purple color is produced.

(2)   To 5 mL of a solution of Phenol for Disinfection (1 in 10,000) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Purity   (1)   Clarity of solution—Dissolve 1.0 g of Phenol for Disinfection in 15 mL of water: the solution is clear.

(2)   Residue on evaporation—Weigh accurately about 5 g of Phenol for Disinfection, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.10% of the mass of the sample.

Assay   Dissolve about 1 g of Phenol for Disinfection, accurately weighed, in water to make exactly 1000 mL. Pipet 25 mL of the solution into an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper immediately, 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.

<div align="center">

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

</div>

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

The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (See the General Notices 5.)

Phenolated Water

フェノール水

Phenolated Water contains not less than 1.8 w/v% and not more than 2.3 w/v% of phenol (C_6H_6O: 94.11).

Method of preparation

Liquefied Phenol	22 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Mix the above ingredients.

Description   Phenolated Water is a colorless, clear liquid, having the odor of phenol.

Identification   **(1)**   Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water: a blue-purple color develops.

(2)   To 5 mL of a solution of Phenolated Water (1 in 200) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Assay   Take exactly 2 mL of Phenolated Water into an iodine flask, add 25 mL of water, then add exactly 40 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes, and allow to stand for 15 minutes. Add 7 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.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

Containers and storage   Containers—Tight containers.

Phenolated Water for Disinfection

消毒用フェノール水

Phenolated Water for Disinfection contains not less than 2.8 w/v% and not more than 3.3 w/v% of phenol (C_6H_6O: 94.11).

Method of preparation

Phenol for Disinfection	31 g
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Mix the above ingredients.

Description   Phenolated Water for Disinfection is a clear, colorless liquid, having the odor of phenol.

Identification   **(1)**   Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water for Disinfection: a blue-purple color develops.

(2)   Proceed with 5 mL of a solution of Phenolated Water for Disinfection (1 in 200) as directed in the Identification (2) under Phenol for Disinfection.

Assay   Take exactly 5 mL of Phenolated Water for Disinfection, add water to make exactly 100 mL, then pipet 25 mL of the solution into an iodine flask, and proceed as directed in the Assay under Phenol for Disinfection.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

Containers and storage   Containers—Tight containers.

Dental Phenol with Camphor

歯科用フェノール・カンフル

Method of preparation

Phenol	35 g
d- or *dl*-Camphor	65 g
	To make 100 g

Melt Phenol by warming, add *d*-Camphor or *dl*- Camphor, and mix.

Description   Dental Phenol with Camphor is a colorless or light red liquid. It has a characteristic odor.

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

Phenol and Zinc Oxide Liniment

フェノール・亜鉛華リニメント

Method of preparation

Liquefied Phenol	22 mL
Powdered Tragacanth	20 g
Carmellose Sodium	30 g
Glycerin	30 mL
Zinc Oxide	100 g
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 g

Mix Liquefied Phenol, Glycerin and Purified Water or Purified Water in Containers, add Powdered Tragacanth in small portions by stirring, and allow the mixture to stand overnight. To the mixture add Carmellose Sodium in small portions by stirring to make a pasty mass, add Zinc Oxide in small portions, and mix. Less than 5 g of Powdered Tragacanth or Carmellose Sodium can be replaced by each other to make 50 g in total.

Description   Phenol and Zinc Oxide Liniment is a white, pasty mass. It has a slight odor of phenol.

Identification   **(1)**   Shake well 1 g of Phenol and Zinc Oxide Liniment with 10 mL of diethyl ether, and filter. To the filtrate add 10 mL of dilute sodium hydroxide TS, shake well, and separate the water layer. To 1 mL of the water layer add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and add 3 mL of sodium hydroxide TS: a yellow color develops (phenol).

(2)   Place 1 g of Phenol and Zinc Oxide Liniment in a porcelain crucible, heat gradually raising the temperature until the content is charred, and then ignite it strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is

produced (zinc oxide).

(3) Shake 0.5 g of Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, separate the chloroform layer, and use this solution as the sample solution. Separately, dissolve 0.01 g of phenol 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 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 (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: the spots obtained from the sample solution and the standard solution show the same *R*f value.

Containers and storage   Containers—Tight containers.

Phenolsulfonphthalein

フェノールスルホンフタレイン
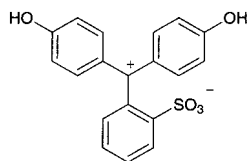
$C_{19}H_{14}O_5S$: 354.38
2-[Bis(4-hydroxyphenyl)methyliumyl]benzenesulfonate
[*143-74-8*]

Phenolsulfonphthalein, when dried, contains not less than 98.0% of phenolsulfonphthalein ($C_{19}H_{14}O_5S$).

Description   Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.
It is very slightly soluble in water and in ethanol (95).
It dissolves in sodium hydroxide TS.

Identification   **(1)** Dissolve 5 mg of Phenolsulfonphthalein in 2 to 3 drops of sodium hydroxide TS, add 2 mL of 0.05 mol/L bromine VS and 1 mL of dilute sulfuric acid, shake well, and allow to stand for 5 minutes. Render the solution alkaline with sodium hydroxide TS: a deep blue-purple color develops.

(2) Dissolve 0.01 g of Phenolsulfonphthalein in diluted sodium carbonate TS (1 in 10) to make 200 mL. To 5 mL of this solution add diluted sodium carbonate TS (1 in 10) to make 100 mL. Perform the test with 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.

Purity   **(1)** Insoluble substances—To about 1 g of Phenolsulfonphthalein, accurately weighed, add 20 mL of a solution of sodium hydrogen carbonate (1 in 40). Allow the mixture to stand for 1 hour with frequent shaking, dilute with water to 100 mL, and allow to stand for 24 hours. Collect the insoluble substances using a tared glass filter (G4), wash with 25 mL of a solution of sodium hydrogen carbonate (1 in 100) and with five 5-mL portions of water, and dry at 105°C for 1 hour: the mass of the residue is not more than 0.2%.

(2) Related substances—Dissolve 0.10 g of Phenolsulfonphthalein in 5 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add dilute sodium hydroxide TS 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 *t*-amyl alcohol, acetic acid (100) and water (4:1:1) to a distance of about 15 cm, and air-dry the plate. After allowing the plate to stand in an ammonia vapor, 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, silica gel, 4 hours).

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

Assay   Weigh accurately about 0.15 g of Phenolsulfonphthalein, previously dried, transfer to an iodine flask, dissolve in 30 mL of a solution of sodium hydroxide (1 in 250), and add water to make 200 mL. Add exactly measured 50 mL of 0.05 mol/L bromine VS, add 10 mL of hydrochloric acid to the solution quickly, and stopper immediately. Allow the mixture to stand for 5 minutes with occasional shaking, add 7 mL of potassium iodide TS, stopper again immediately, and shake gently for 1 minute. Titrate <2.50> the liberated 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 bromine VS
= 4.430 mg of $C_{19}H_{14}O_5S$

Containers and storage   Containers—Well-closed containers.

Phenolsulfonphthalein Injection

フェノールスルホンフタレイン注射液

Phenolsulfonphthalein Injection is an aqueous injection.
It contains not less than 0.54 w/v% and not more than 0.63 w/v% of phenolsulfonphthalein ($C_{19}H_{14}O_5S$: 354.38).

Method of preparation

Phenolsulfonphthalein	6 g
Sodium Chloride	9 g
Sodium Bicarbonate	1.43 g
(or Sodium Hydroxide	0.68 g)
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
To make	1000 mL

Prepare as directed under Injections, with the above ingredients.

Description   Phenolsulfonphthalein Injection is a clear, orange-yellow to red liquid.

Identification   To 1 mL of Phenolsulfonphthalein Injection add 2 to 3 drops of sodium hydroxide TS, and proceed as directed in the Identification (1) under Phenolsulfonphthalein.

pH <2.54>   6.0 – 7.6

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.

Sensitivity To 1.0 mL of Phenolsulfonphthalein Injection add 5 mL of water. To 0.20 mL of this solution add 50 mL of freshly boiled and cooled water and 0.40 mL of 0.01 mol/L sodium hydroxide VS: a deep red-purple color develops, and it changes to light yellow on the addition of 0.40 mL of 0.005 mol/L sulfuric acid VS.

Assay Pipet 5 mL of Phenolsulfonphthalein Injection, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of phenolsulfonphthalein for assay, previously dried in a desiccator (silica gel) for 4 hours, and dissolve in a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 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 559 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of phenolsulfonphthalein ($C_{19}H_{14}O_5S$)
  $= M_S \times A_T/A_S$

M_S: Amount (mg) of phenolsulfonphthalein for assay taken

Containers and storage Containers—Hermetic containers.

L-Phenylalanine

L-フェニルアラニン
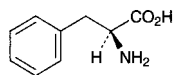
$C_9H_{11}NO_2$: 165.19
(2S)-2-Amino-3-phenylpropanoic acid
[63-91-2]

L-Phenylalanine, when dried, contains not less than 98.5% of L-phenylalanine ($C_9H_{11}NO_2$).

Description L-Phenylalanine 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-Phenylalanine, 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> $[\alpha]_D^{20}$: −33.0 – −35.5° (after drying, 0.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of L-Phenylalanine in 20 mL of water: the pH of this solution is between 5.3 and 6.3.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Phenylalanine 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-Phenylalanine. 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-Phenylalanine. 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-Phenylalanine. 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-Phenylalanine 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>—Dissolve 1.0 g of L-Phenylalanine in 5 mL of dilute hydrochloric acid and 15 mL of water, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Phenylalanine 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 a solution of ninhydrin in acetone (1 in 50) on the plate, 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 0.17 g of L-Phenylalanine, 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
  = 16.52 mg of $C_9H_{11}NO_2$

Containers and storage Containers—Tight containers.

Phenylbutazone

フェニルブタゾン
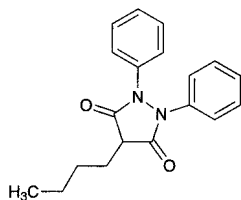
C$_{19}$H$_{20}$N$_2$O$_2$: 308.37
4-Butyl-1,2-diphenylpyrazolidine-3,5-dione
[*50-33-9*]

Phenylbutazone, when dried, contains not less than 99.0% of phenylbutazone (C$_{19}$H$_{20}$N$_2$O$_2$).

Description   Phenylbutazone occurs as a white to slightly yellowish white, crystalline powder. It is odorless, and is at first tasteless but leaves a slightly bitter aftertaste.

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

It dissolves in sodium hydroxide TS.

Identification   **(1)**   To 0.1 g of Phenylbutazone add 1 mL of acetic acid (100) and 1 mL of hydrochloric acid, and heat on a water bath under a reflux condenser for 30 minutes. Add 10 mL of water, and cool with ice water. Filter, and to the filtrate add 3 to 4 drops of sodium nitrite TS. To 1 mL of this solution add 1 mL of 2-naphthol TS and 3 mL of chloroform, and shake: a deep red color develops in the chloroform layer.

(2)   Dissolve 1 mg of Phenylbutazone in 10 mL of dilute sodium hydroxide TS, and dilute 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: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60>   104 – 107°C

Purity   **(1)**   Clarity of solution—Dissolve 1.0 g of Phenylbutazone in 20 mL of sodium hydroxide solution (2 in 25), and allow to stand at 25 ± 1°C for 3 hours: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.05.

(2)   Heavy metals <1.07>—Proceed with 2.0 g of Phenylbutazone 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 phenylbutazone, according to Method 3, and perform the test (not more than 2 ppm).

(4)   Readily carbonizable substances—Dissolve 1.0 g of Phenylbutazone in 20 mL of sulfuric acid, and allow to stand at 25 ± 1°C for exactly 30 minutes: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.10.

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 0.1% (1 g).

Assay   Weigh accurately about 0.5 g of Phenylbutazone,

previously dried, dissolve in 25 mL of acetone, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the solution shows a blue color which persists for 15 seconds (indicator: 5 drops of bromothymol blue TS). Perform a blank determination with a mixture of 25 mL of acetone and 16 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 30.84 mg of C$_{19}$H$_{20}$N$_2$O$_2$

Containers and storage   Containers—Tight containers.

Phenylephrine Hydrochloride

フェニレフリン塩酸塩
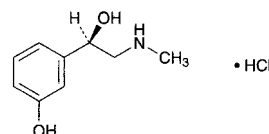
C$_9$H$_{13}$NO$_2$.HCl: 203.67
(1*R*)-1-(3-Hydroxyphenyl)-2-methylaminoethanol monohydrochloride
[*61-76-7*]

Phenylephrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of phenylephrine hydrochloride (C$_9$H$_{13}$NO$_2$.HCl).

Description   Phenylephrine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

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

The pH of a solution of 1.0 g of Phenylephrine Hydrochloride in 100 mL of water is 4.5 to 5.5.

Identification   **(1)**   To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue color is produced. To the solution so obtained add 1 mL of diethyl ether, and shake vigorously: no blue color develops in the diethyl ether layer.

(2)   To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of iron (III) chloride TS: a persistent purple color is produced.

(3)   Dissolve 0.3 g of Phenylephrine Hydrochloride in 3 mL of water, add 1 mL of ammonia TS, and rub the inner side of the test tube with a glass rod: a precipitate is produced. Collect the precipitate, wash with a few drops of ice-cold water, and dry at 105°C for 2 hours: it melts <2.60> between 170°C and 177°C.

(4)   A solution of Phenylephrine Hydrochloride (1 in 100) responds to Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49>   [α]$_D^{20}$: −42.0 – −47.5° (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point <2.60>   140 – 145°C

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

(2)   Sulfate <1.14>—Take 0.5 g of Phenylephrine Hydrochloride, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3)   Ketone—Dissolve 0.20 g of Phenylephrine Hydro-

chloride in 1 mL of water, and add 2 drops of sodium penta-cyanonitrosylferrate (III) TS, 1 mL of sodium hydroxide TS and then 0.6 mL of acetic acid (100): the solution has no more color than the following control solution.

Control solution: Prepare as directed above without Phenylephrine Hydrochloride.

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

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

Assay   Weigh accurately about 0.1 g of Phenylephrine Hydrochloride, previously dried, dissolve in 40 mL of water contained in an iodine flask, add exactly measured 50 mL of 0.05 mol/L bromine VS, then add 5 mL of hydrochloric acid, and immediately stopper tightly. Shake the mixture, and allow to stand for 15 minutes. To this solution add 10 mL of potassium iodide TS carefully, stopper tightly immediately, shake thoroughly, allow to stand for 5 minutes, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Each mL of 0.05 mol/L bromine VS}$$
$$= 3.395 \text{ mg of } C_9H_{13}NO_2.HCl$$

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

Phenytoin

Diphenylhydantoin

フェニトイン
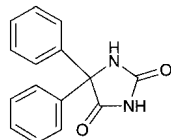
C$_{15}$H$_{12}$N$_2$O$_2$: 252.27
5,5-Diphenylimidazolidine-2,4-dione
[*57-41-0*]

Phenytoin, when dried, contains not less than 99.0% of phenytoin (C$_{15}$H$_{12}$N$_2$O$_2$).

Description   Phenytoin occurs as a white, crystalline powder or granules. It is odorless and tasteless.

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

It dissolves in sodium hydroxide TS.

Melting point: about 296°C (with decomposition).

Identification   (1)   Dissolve 0.02 g of Phenytoin in 2 mL of ammonia TS, and add 5 mL of silver nitrate TS: a white precipitate is produced.

(2)   Boil a mixture of 0.01 g of Phenytoin, 1 mL of ammonia TS and 1 mL of water, and add dropwise 2 mL of a mixture prepared from 50 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 10 mL of ammonia TS: a red, crystalline precipitate is produced.

(3)   Heat 0.1 g of Phenytoin with 0.2 g of sodium hydroxide, and fuse: the gas evolved turns moistened red litmus paper blue.

(4)   Add 3 mL of chlorinated lime TS to 0.1 g of Phenytoin, shake for 5 minutes, and dissolve the oily pre-

cipitate in 15 mL of hot water. After cooling, add 1 mL of dilute hydrochloric acid dropwise, then add 4 mL of water. Filter the white precipitate thus obtained, wash with water, and press it with dry filter paper to remove the accompanying water. Dissolve the precipitate with 1 mL of chloroform, add 5 mL of diluted ethanol (9 in 10), and rub the inner surface of the flask to produce a white, crystalline precipitate. Collect the precipitate, wash with ethanol (95), and dry: the melting point <2.60> is between 165°C and 169°C.

Purity   (1)   Clarity and color of solution—Dissolve 0.20 g of Phenytoin in 10 mL of 0.2 mol/L sodium hydroxide VS: the solution is clear and colorless. Then heat the solution: no turbidity is produced. Cool, and mix the solution with 5 mL of acetone: the solution is clear and colorless.

(2)   Acidity or alkalinity—Shake 2.0 g of Phenytoin with 40 mL of water for 1 minute, filter, and perform the following tests using this filtrate as the sample solution.

(i)   To 10 mL of the sample solution add 2 drops of phenolphthalein TS: no color develops. Then add 0.15 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(ii)   To 10 mL of the sample solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and 5 drops of methyl red TS: a red to orange color develops.

(3)   Chloride <1.03>—Dissolve 0.30 g of Phenytoin in 30 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 from 0.60 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of acetone and 6 mL of dilute nitric acid, and add water to 50 mL (not more than 0.071%).

(4)   Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin 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% (2 g, 105°C, 2 hours).

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

Assay   Weigh accurately about 0.5 g of Phenytoin, previously dried, dissolve in 40 mL of ethanol (95) with the aid of gentle heating, add 0.5 mL of thymolphthalein TS immediately, and titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops. Then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 25 mL of silver nitrate TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a light red color, which persists for 1 minute, develops.

$$\text{Each mL of 0.1 mol/L sodium hydroxide VS}$$
$$= 25.23 \text{ mg of } C_{15}H_{12}N_2O_2$$

Containers and storage   Containers—Well-closed containers.

Phenytoin Powder

Diphenylhydantoin Powder

フェニトイン散

Phenytoin Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin (C$_{15}$H$_{12}$N$_2$O$_2$: 252.27).

Method of preparation   Prepare as directed under Granules or Powders, with Phenytoin.

Identification Weigh a portion of Phenytoin Powder, equivalent to 0.3 g of Phenytoin, stir well with two 100-mL portions of diethyl ether, and extract. Combine the diethyl ether extracts, and filter. Evaporate the filtrate on a water bath to dryness, and proceed with the residue as directed in the Identification under Phenytoin.

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

Assay Weigh accurately an amount of Phenytoin Powder, equivalent to about 50 mg of phenytoin ($C_{15}H_{12}N_2O_2$), add 30 mL of methanol, treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, 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 25 mg of phenytoin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 25 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 phenytoin to that of the internal standard.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2)$$
$$= M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of phenytoin for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 258 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 0.02 mol/L phosphate buffer solution (pH 3.5) (11:9).
Flow rate: Adjust so that the retention time of phenytoin 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, phenytoin 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 ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Phenytoin Tablets

Diphenylhydantoin Tablets

フェニトイン錠

Phenytoin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

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

Identification Weigh a portion of powdered Phenytoin Tablets, equivalent to about 0.3 g of Phenytoin, transfer to a separator, and add 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with 100 mL of diethyl ether, then with four 25-mL potions of diethyl ether. Combine the extracts, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours. Proceed with the residue as directed in the Identification under Phenytoin.

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 Phenytoin Tablets add $3V/5$ mL of a mixture of water and acetonitrile (1:1), treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly V mL so that each mL contains about 1 mg of phenytoin ($C_{15}H_{12}N_2O_2$). Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2)$$
$$= M_S \times Q_T/Q_S \times V/25$$

M_S: Amount (mg) of phenytoin for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).

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

Assay Weigh accurately the mass of not less than 20 Phenytoin Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 50 mg of phenytoin ($C_{15}H_{12}N_2O_2$), add 30 mL of a mixture of water and acetonitrile (1:1), treat with ultrasound waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Centrifuge this solution, 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 25 mg of phenytoin for assay, previously dried at 105°C for 2 hours, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 25 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 phenytoin to that of the internal standard.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2)$$
$$= M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of phenytoin for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 258 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 0.02 mol/L phosphate buffer solution (pH 3.5) (11:9).
Flow rate: Adjust so that the retention time of phenytoin 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, phenytoin 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 ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

注射用フェニトインナトリウム
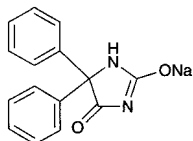
C$_{15}$H$_{11}$N$_2$NaO$_2$: 274.25
Monosodium 5,5-diphenyl-4-oxoimidazolidin-2-olate
[*630-93-3*]

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.
When dried, it contains not less than 98.5% of phenytoin sodium (C$_{15}$H$_{11}$N$_2$NaO$_2$), and contains not less than 92.5% and not more than 107.5% of the labeled amount of phenytoin sodium (C$_{15}$H$_{11}$N$_2$NaO$_2$).

Method of preparation   Prepare as directed under Injections.

Description   Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless.
It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether.
The pH of a solution of 1.0 g of Phenytoin Sodium for Injection in 20 mL of water is about 12.
It is hygroscopic.
A solution of Phenytoin Sodium for Injection absorbs carbon dioxide gradually when exposed to air, and a crystalline precipitate of phenytoin is produced.

Identification   (1)   With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.
(2)   Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <*1.09*> (1) for sodium salt.

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.
(2)   Heavy metals <*1.07*>—Proceed with 1.0 g of Phenytoin Sodium for Injection 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 2.5% (1 g, 105°C, 4 hours).

Assay   Weigh accurately the content of not less than 10 containers of Phenytoin Sodium for Injection, transfer about 0.3 g of the content, previously dried and accurately weighed, to a separator, dissolve in 50 mL of water, add 10 mL of dilute hydrochloric acid, and extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate on a water bath. Dry the residue at 105°C for 2 hours, and weigh it as the mass of phenytoin (C$_{15}$H$_{12}$N$_2$O$_2$: 252.27).

Amount (mg) of phenytoin sodium (C$_{15}$H$_{11}$N$_2$NaO$_2$)
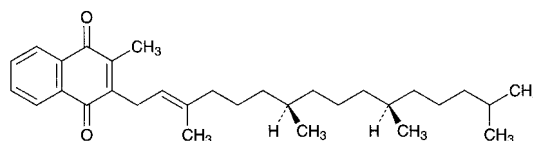= amount (mg) of phenytoin (C$_{15}$H$_{12}$N$_2$O$_2$) × 1.087

Containers and storage   Containers—Hermetic containers.

Phytonadione

Phytomenadione
Vitamin K$_1$

フィトナジオン

C$_{31}$H$_{46}$O$_2$: 450.70
2-Methyl-3-[(2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]-1,4-naphthoquinone
[*84-80-0*]

Phytonadione contains not less than 97.0% and not more than 102.0% of phytonadione (C$_{31}$H$_{46}$O$_2$).

Description   Phytonadione is a clear yellow to orange-yellow, viscous liquid.
It is miscible with isooctane.
It is soluble in ethanol (99.5), and practically insoluble in water.
It decomposes gradually and changes to a red-brown by light.
Specific gravity d_{20}^{20}: about 0.967

Identification   (1)   Determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 100,000) 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 Phytonadione in isooctane (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.

(2) Determine the infrared absorption spectrum of Phytonadione 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}: 1.525 – 1.529

Purity (1) Ratio of absorbances—Determine the absorbances, A_1, A_2 and A_3, of a solution of Phytonadione in isooctane (1 in 100,000) at 248.5 nm, 253.5 nm and 269.5 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>: the ratio A_2/A_1 is between 0.69 and 0.73, and the ratio A_2/A_3 is between 0.74 and 0.78. Determine the absorbances, A_4 and A_5, of a solution of Phytonadione in isooctane (1 in 10,000) at 284.5 nm and 326 nm, respectively: the ratio A_4/A_5 is between 0.28 and 0.34.

(2) Heavy metals <1.07>—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed 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) Menadione—Dissolve 20 mg of Phytonadione in 0.5 mL of a mixture of water and ethanol (95) (1:1), add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

Isomer ratio Conduct this procedure rapidly and without exposure to light. Dissolve 30 mg of Phytonadione in 50 mL of the mobile phase. To 4 mL of this solution add the mobile phase to make 25 mL. To 10 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Perform the test with 50 µL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of Z-isomer and E-isomer, A_{TZ} and A_{TE}: $A_{TZ}/(A_{TZ} + A_{TE})$ is between 0.05 and 0.18.

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 sample solution under the above operating conditions, Z-isomer and E-isomer 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 50 µL of the sample solution under the above operating conditions, the relative standard deviation of the total area of the peaks of Z-isomer and E-isomer is not more than 2.0%.

Assay Conduct this procedure rapidly and without exposure to light. Weigh accurately about 30 mg each of Phytonadione and Phytonadione RS, and dissolve each in the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, and add the mobile phase to make exactly 25 mL. To exactly 10 mL each of these solutions add exactly 7 mL of the internal standard solution and the mobile phase to make 25 mL, and use these 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 total area of the peaks of Z-isomer and E-isomer to the peak area of the internal standard.

Amount (mg) of phytonadione $(C_{31}H_{46}O_2) = M_S \times Q_T/Q_S$

M_S: Amount (mg) of Phytonadione RS taken

Internal standard solution—A solution of cholesterol benzoate in the mobile phase (1 in 400).
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 porous 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 and n-amyl alcohol (4000 : 3).

Flow rate: Adjust so that the retention time of the peak of E-isomer of phytonadione is about 25 minutes.

System suitability—

System performance: When the procedure is run with 50 µL of the standard solution under the above operating conditions, the internal standard, Z-isomer and E-isomer are eluted in this order with the resolution between the peaks of Z-isomer and E-isomer being not less than 1.5.

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 total area of the peaks of Z-isomer and E-isomer to the peak area of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a cold place or in containers in which air has been displaced by Nitrogen.

Pilocarpine Hydrochloride

ピロカルピン塩酸塩
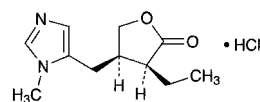
C$_{11}$H$_{16}$N$_2$O$_2$.HCl: 244.72
(3S,4R)-3-Ethyl-4-(1-methyl-1H-imidazol-5-ylmethyl)-4,5-dihydrofuran-2(3H)-one monohydrochloride
[54-71-7]

Pilocarpine Hydrochloride, when dried, contains not less than 99.0% of pilocarpine hydrochloride (C$_{11}$H$_{16}$N$_2$O$_2$.HCl).

Description Pilocarpine Hydrochloride occurs as colorless crystals or white powder. It is odorless, and has a slightly bitter taste.

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

The pH of a solution of 1.0 g of Pilocarpine Hydrochloride in 10 mL of water is between 3.5 and 4.5.

It is hygroscopic.

It is affected by light.

Identification (1) Dissolve 0.1 g of Pilocarpine Hydrochloride in 5 mL of water, add 1 drop of dilute nitric acid, 1 mL of hydrogen peroxide TS, 1 mL of chloroform and 1 drop of a potassium dichromate solution (1 in 300), and shake the mixture vigorously: a violet color develops in the chloroform layer while no color or a light yellow color is produced in the aqueous layer.

(2) To 1 mL of a solution of Pilocarpine Hydrochloride (1 in 20) add 1 mL of dilute nitric acid and 2 to 3 drops of silver nitrate TS: a white precipitate or opalescence is produced.

Melting point <2.60>   200 – 203°C

Purity (1) Sulfate—Dissolve 0.5 g of Pilocarpine Hydrochloride in 20 mL of water, and use this solution as the sample solution. To 5.0 mL of the sample solution add 1 mL of dilute hydrochloric acid and 0.5 mL of barium chloride TS: no turbidity is produced.

(2) Nitrate—To 2.0 mL of the sample solution obtained in (1) add 2 mL of iron (II) sulfate TS, and superimpose the mixture upon 4 mL of sulfuric acid: no dark brown color develops at the zone of contact.

(3) Related substances—Dissolve 0.3 g of Pilocarpine 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 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, methanol and ammonia TS (85:14:2) to a distance of about 13 cm, and dry the plate at 105°C for 10 minutes. Cool, and spray evenly bismuth potassium iodide 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.

(4) Readily carbonizable substances <1.15>—Take 0.25 g of Pilocarpine Hydrochloride, and perform the test: the solution has no more color than Matching Fluid B.

Loss on drying <2.41>   Not more than 3.0% (1 g, 105°C, 2 hours).

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

Assay Weigh accurately about 0.5 g of Pilocarpine 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
= 24.47 mg of $C_{11}H_{16}N_2O_2$.HCl

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

Pilocarpine Hydrochloride Tablets

ピロカルピン塩酸塩錠

Pilocarpine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.HCl: 244.72).

Method of preparation Prepare as directed under Tablets, with Pilocarpine Hydrochloride.

Identification Perform the test with 10 μL each of the sample solution and the standard solution, both obtained in the assay, as directed under Liquid Chromatography <2.01> according to the following conditions: the principal peaks in the chromatograms obtained from the sample solution and standard solution show the same retention time, and both spectra of these peaks in the chromatograms exhibit similar intensities of absorption at the same wavelengths.
Operating conditions—
Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Detector: Photodiode array detector (wavelength: 215 nm; spectrum range of measurement: 200 – 370 nm).
System suitability—
System performance: Proceed as directed in the system suitability in the Assay.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add phosphate buffer solution (pH 4.0) 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 area of the two peaks, having the relative retention time of about 0.78 and about 0.92 to pilocarpine, obtained from the sample solution is not larger than the peak area of pilocarpine obtained from the standard solution, the area of the peak other than pilocarpine and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of pilocarpine from the standard solution, and the total area of the peaks other than pilocarpine from the sample solution is not larger than 2 times the peak area of pilocarpine 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 1.3 times as long as the retention time of pilocarpine, beginning after the solvent peak.
System suitability—
Test for required detectability: To exactly 2 mL of the standard solution add phosphate buffer solution (pH 4.0) to make exactly 20 mL. Confirm that the peak area of pilocarpine obtained with 10 μL of this solution is equivalent to 7 – 13% 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 pilocarpine are not less than 3000 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 pilocarpine 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 Pilocarpine Hydrochloride Tablets add a suitable amount of phosphate buffer solution (pH 4.0), shake until the tablet is completely disintegrated, then add phosphate buffer solution (pH 4.0) to make exactly V mL so that each mL contains about 0.2 mg of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2.HCl$), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of pilocarpine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in phosphate buffer solution (pH 4.0) to make exactly 100 mL. Pipet 5 mL of this solution, add phosphate buffer solution (pH 4.0) 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_T and A_S, of pilocarpine in each solution.

$$\text{Amount (mg) of pilocarpine hydrochloride}$$
$$(C_{11}H_{16}N_2O_2.HCl)$$
$$= M_S \times A_T/A_S \times V/200$$

M_S: Amount (mg) of pilocarpine 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 pilocarpine 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 pilocarpine 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 30 minutes of Pilocarpine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pilocarpine Hydrochloride Tablets, 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 3 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 pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of pilocarpine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium 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 pilocarpine in each solution.

Dissolution rate (%) with respect to the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2.HCl$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$$

M_S: Amount (mg) of pilocarpine hydrochloride for assay taken
C: Labeled amount (mg) of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2.HCl$) 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 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pilocarpine 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 pilocarpine is not more than 1.0%.

Assay To 20 Pilocarpine Hydrochloride Tablets add a suitable amount of phosphate buffer solution (pH 4.0), shake until the tablets are completely disintegrated, then add phosphate buffer solution (pH 4.0) to make exactly V mL so that each mL contains about 0.4 mg of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2.HCl$), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of pilocarpine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in phosphate buffer solution (pH 4.0) 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 the peak areas, A_T and A_S, of pilocarpine in each solution.

$$\text{Amount (mg) of pilocarpine hydrochloride}$$
$$(C_{11}H_{16}N_2O_2.HCl) \text{ in 1 tablet}$$
$$= M_S \times A_T/A_S \times V/2000$$

M_S: Amount (mg) of pilocarpine hydrochloride for assay taken

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 215 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with phenylated silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust to pH 2.5. To this solution add 5.0 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid.
Flow rate: Adjust so that the retention time of pilocarpine 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, the number of theoretical plates and the symmetry

factor of the peak of pilocarpine are not less than 3000 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 pilocarpine is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Pilsicainide Hydrochloride Hydrate

ピルシカイニド塩酸塩水和物
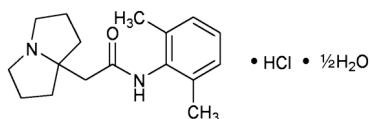
C$_{17}$H$_{24}$N$_2$O.HCl. $^1\!/_2$H$_2$O: 317.85
N-(2,6-Dimethylphenyl)tetrahydro-1*H*-pyrrolizin-7a(5*H*)-ylacetamide monohydrochloride hemihydrate
[*88069-49-2*, anhydride]

Pilsicainide Hydrochloride Hydrate contains not less than 99.0% and not more than 101.0% of pilsicainide hydrochloride hydrate (C$_{17}$H$_{24}$N$_2$O.HCl. $^1\!/_2$H$_2$O).

Description   Pilsicainide Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is very soluble in acetic acid (100), and freely soluble in water, in methanol and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification   (1)   Determine the absorption spectrum of a solution of Pilsicainide Hydrochloride Hydrate in 0.1 mol/L hydrochloric acid TS (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 Pilsicainide 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.

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

pH <2.54>   Dissolve 1.0 g of Pilsicainide Hydrochloride Hydrate in 50 mL of water: the pH of this solution is between 5.3 and 6.1.

Melting point <2.60>   210.5 – 213.5°C (Heat the bath to 160°C in advance).

Purity   (1)   Heavy metals <1.07>—Proceed with 2.0 g of Pilsicainide 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).

(2)   Related substances—Dissolve 40 mg of Pilsicainide Hydrochloride Hydrate in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 20 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 exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> ac-

cording to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than pilsicainide obtained from the sample solution is not larger than the peak area of pilsicainide 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 40°C.

Mobile phase: To 750 mL of water add 5 mL of triethylamine, adjust to pH 4.0 with phosphoric acid, and add water to make 1000 mL. To this solution add 200 mL of acetonitrile for liquid chromatography.

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

Time span of measurement: About 5 times as long as the retention time of pilsicainide, 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 pilsicainide 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 pilsicainide is not more than 2.0%.

Water <2.48>   2.5 – 3.3% (50 mg, coulometric titration).

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

Assay   Weigh accurately about 0.3 g of Pilsicainide Hydrochloride Hydrate, dissolve it 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.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 31.79 \text{ mg of } C_{17}H_{24}N_2O.HCl.\, ^1\!/_2H_2O$$

Containers and storage   Containers—Tight containers.

Pilsicainide Hydrochloride Capsules

ピルシカイニド塩酸塩カプセル

Pilsicainide Hydrochloride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of pilsicainide hydrochloride hydrate (C$_{17}$H$_{24}$N$_2$O.HCl. $^1\!/_2$H$_2$O: 317.85).

Method of preparation   Prepare as directed under Capsules, with Pilsicainide Hydrochloride Hydrate.

Identification   Take out the contents of Pilsicainide Hydrochloride Capsules, to a quantity of the content, equivalent to 50 mg of Pilsicainide Hydrochloride Hydrate, add 10 mL of water, and shake well. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To 1 mL of the filtrate, add 1

mL of 1 mol/L hydrochloric acid TS and 8 mL of water. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 261 nm and 265 nm, and between 268 nm and 272 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 capsule of Pilsicainide Hydrochloride Capsules, add water, and shake to disperse the content of the capsule uniformly while warming in a water bath. After cooling, add exactly V mL of the internal standard solution so that 0.2 mL of the internal standard solution is added for each mg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$), then, add water so that each mL contains about 0.5 mg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$). To 5 mL of this solution, add water to make 50 mL, and filter. 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 pilsicainide hydrochloride hydrate
> ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$)
> $= M_S \times Q_T/Q_S \times V/10$

M_S: Amount (mg) of pilsicainide hydrochloride hydrate for assay taken

Internal Standard Solution—Dissolve 2.5 g of lidocaine for assay in 20 mL of 0.5 mol/L hydrochloric acid TS, and add water to make 1000 mL.

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 Pilsicainide Hydrochloride Capsules is not less than 85%.

Start the test with 1 capsule of Pilsicainide Hydrochloride 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 28 μg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of pilsicainide hydrochloride hydrate for assay, 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 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 pilsicainide in each solution.

> Dissolution rate (%) with respect to the labeled amount of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$)
> $= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of pilsicainide hydrochloride hydrate for assay taken
C: Labeled amount (mg) of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$) 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 con-

ditions, the number of theoretical plates and the symmetry factor of the peak of pilsicainide 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 pilsicainide is not more than 1.0%.

Assay Take out the contents of not less than 20 Pilsicainide Hydrochloride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$), add 50 mL of water and shake well. After adding exactly 10 mL of the internal standard solution, add water to make 100 mL. To 5 mL of this solution add water to make 50 mL, and filter the solution. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of pilsicainide hydrochloride hydrate for assay, dissolve in exactly 10 mL of the internal standard solution, and add water to make 100 mL. To 5 mL of this solution add water 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 pilsicainide to that of the internal standard.

> Amount (mg) of pilsicainide hydrochloride hydrate
> ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$)
> $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of pilsicainide hydrochloride hydrate for assay taken

Internal Standard Solution—Dissolve 2.5 g of lidocaine for assay in 20 mL of 0.5 mol/L hydrochloric acid TS, and add water to make 1000 mL.
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 around 40°C.
Mobile phase: To 750 mL of water add 5 mL of triethylamine, adjust the pH to 4.0 with phosphoric acid, and add water to make 1000 mL. To this solution, add 200 mL of acetonitrile for liquid chromatography.
Flow rate: Adjust so that the retention time of pilsicainide 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 and pilsicainide are eluted in this order with the resolution between these peaks being not less than 2.0.
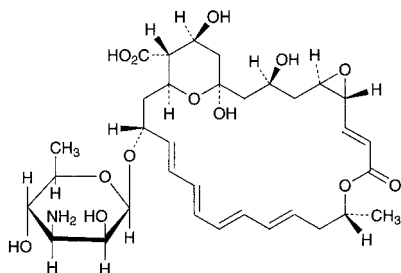System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pilsicainide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pimaricin

Natamycin

ピマリシン

C_{33}H_{47}NO_{13}: 665.73
(1*R**,3*S**,5*R**,7*R**,8*E*,12*R**,14*E*,16*E*,18*E*,20*E*,22*R**,
24*S**,25*R**,26*S**)-22-(3-Amino-3,6-dideoxy-*β*-D-
mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-
6,11,28-trioxatricyclo[22.3.1.0^{5,7}]octacosa-8,14,16,18,20-
pentaene-25-carboxylic acid
[*7681-93-8*]

Pimaricin is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces natalensis*.

It contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pimaricin is expressed as mass (potency) of pimaricin (C_{33}H_{47}NO_{13}).

Description Pimaricin occurs as white to yellowish white crystalline powder.

It is slightly soluble in methanol and in acetic acid (100), and practically insoluble in water and in ethanol (99.5).

Identification (1) To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and mix: a blue-purple color appears.

(2) Dissolve 5 mg of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. 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 Pimaricin 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> [α]_D^{20}: +243 – +259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pimaricin 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).

(2) Related substances—Dissolve 20 mg of Pimaricin in methanol 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, and determine the total area of the peaks other than pimaricin by the automatic integration method. Calculate the amount of the peaks by the area percentage method: not more than 4.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 303 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 40°C.

Mobile phase: Dissolve 1.0 g of ammonium acetate in 1000 mL of a mixture of water, methanol and tetrahydrofuran (47:44:2).

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

Time span of measurement: About 3 times as long as the retention time of pimaricin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add methanol 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 methanol to make exactly 10 mL. Confirm that the peak area of pimaricin 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 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 pimaricin are not less than 1500 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 pimaricin is not more than 2.0%.

Water <2.48> Between 6.0% and 9.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Pimaricin and Pimaricin RS, equivalent to about 25 mg (potency), and dissolve each in methanol to make exactly 100 mL. Pipet 2 mL each of these solutions, add a solution of acetic acid (100) in methanol (1 in 100) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances at 295.5 nm, A_{T1} and A_{S1}, at 303 nm, A_{T2} and A_{S2}, and at 311 nm, A_{T3} and A_{S3}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount [μg (potency)] of pimaricin (C_{33}H_{47}NO_{13})

$$= M_S \times \frac{A_{T2} - \dfrac{A_{T1} + A_{T3}}{2}}{A_{S2} - \dfrac{A_{S1} + A_{S3}}{2}} \times 1000$$
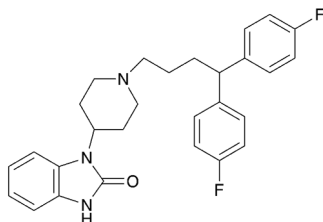
M_S: Amount [mg (potency)] of Pimaricin RS taken

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

Pimozide

ピモジド

C$_{28}$H$_{29}$F$_2$N$_3$O: 461.55
1-{1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl}-
1,3-dihydro-2*H*-benzimidazol-2-one
[*2062-78-4*]

Pimozide contains not less than 98.5% and not more than 101.0% of pimozide (C$_{28}$H$_{29}$F$_2$N$_3$O).

Description Pimozide occurs as a white to pale yellowish white powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Pimozide in methanol (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 Pimozide 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*> 216 – 220°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Pimozide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution by using 5 mL of sulfuric acid (not more than 10 ppm).

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

(3) Related substances—Dissolve 0.10 g of Pimozide 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 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 the peak of pimozide from the sample solution is not larger than the peak area of pimozide from the standard solution, and the total area of the peaks other than the peak of pimozide from the sample solution is not larger than 1.5 times of the peak area of pimozide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 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: Dissolve 2.5 g of ammonium acetate and 8.5 g of tetrabutylammonium hydrogensulfate in water to make 1000 mL.

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	80 → 70	20 → 30
10 – 15	70	30

Flow rate: 2.0 mL per minute.

Time span of measurement: 1.5 times as long as the retention time of pimozide.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of pimozide obtained from 10 μL of this solution is equivalent to 8 to 12% of that of pimozide obtained from 10 μL of the standard solution.

System performance: Dissolve 5 mg of Pimozide and 2 mg of mebendazole in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, mebendazole and pimozide 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 area of pimozide 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 70 mg of Pimozide, previously dried, dissolve in 25 mL of acetic acid for nonaqueous titration, and titrate <*2.50*> with 0.02 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 9.231 mg of C$_{28}$H$_{29}$F$_2$N$_3$O

Containers and storage Containers—Well-closed containers.

Pindolol

ピンドロール

and enantiomer
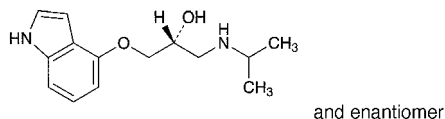
$C_{14}H_{20}N_2O_2$: 248.32
(2*RS*)-1-(1*H*-Indol-4-yloxy)-
3-(1-methylethyl)aminopropan-2-ol
[*13523-86-9*]

Pindolol, when dried, contains not less than 98.5% of pindolol ($C_{14}H_{20}N_2O_2$).

Description Pindolol occurs as a white, crystalline powder. It has a slight, characteristic odor.

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

It dissolves in dilute sulfuric acid and in acetic acid (100).

Identification (1) To 1 mL of a solution of Pindolol in methanol (1 in 10,000) add 1 mL of a solution of 1-(4-pyridyl)-pyridinium chloride hydrochloride (1 in 1000) and 1 mL of sodium hydroxide TS, then add 1 mL of hydrochloric acid: a blue to blue-purple color, changing to red-purple, is produced.

(2) Dissolve 0.05 g of Pindolol in 1 mL of dilute sulfuric acid, and add 1 mL of Reinecke salt TS: a light red precipitate is produced.

(3) Determine the absorption spectrum of a solution of Pindolol 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.

(4) Determine the infrared absorption spectrum of Pindolol, 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> $E_{1cm}^{1\%}$ (264 nm): 333 – 350 (10 mg, methanol, 500 mL).

Melting point <2.60> 169 – 173°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Pindolol in 10 mL of acetic acid (100), and observe immediately: the solution is clear, and has no more color than the following control solution.

Control solution: Measure accurately 4 mL of Matching Fluid A, add exactly 6 mL of water, and mix.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pindolol 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 Pindolol according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pindolol 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. 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, acetone and isopropylamine (5:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (3 in 5) and a sodium nitrite solution (1 in 50) 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, 4 hours).

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

Assay Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS
= 24.83 mg of $C_{14}H_{20}N_2O_2$

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

Pioglitazone Hydrochloride

ピオグリタゾン塩酸塩

and enantiomer
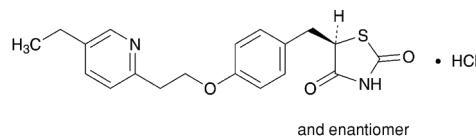
$C_{19}H_{20}N_2O_3S.HCl$: 392.90
(5*RS*)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}thiazolidine-2,4-dione
monohydrochloride
[*112529-15-4*]

Pioglitazone Hydrochloride contains not less than 99.0% and not more than 101.0% of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), calculated on the anhydrous basis.

Description Pioglitazone Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide and in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Pioglitazone Hydrochloride in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Pioglitazone Hydrochloride 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 or the spectrum of a solution of Pioglitazone 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 Pioglitazone 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 Pioglitazone Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Pioglitazone Hydrochloride in 1 mL of nitric acid, and add 4 mL of dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pioglitazone Hydrochloride according to Method 4, and perform the test. After incineration, use 3 mL of hydrobromic acid instead of 3 mL of hydrochloric acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Pioglitazone Hydrochloride in 20 mL of methanol, add the mobile phase to make 100 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 40 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 peaks, having the relative retention times of about 0.7, about 1.4 and about 3.0 to pioglitazone from the sample solution, is not larger than 2/5 times the peak area of pioglitazone from the standard solution, and the area of each peak other than pioglitazone and those peaks mentioned above from the sample solution is smaller than 1/5 times the peak area of pioglitazone from the standard solution. Furthermore, the total area of the peaks other than pioglitazone from the sample solution is not larger than the peak area of pioglitazone 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 4 times as long as the retention time of pioglitazone, 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 pioglitazone obtained from 40 μL of this solution is equivalent to 7 to 13% of that of pioglitazone obtained from 40 μL of the standard solution.

System performance: Dissolve 50 mg of Pioglitazone Hydrochloride in 10 mL of a solution of benzophenone in methanol (1 in 750), and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 40 μL of this solution under the above operating conditions, pioglitazone and benzophenone 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 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 2.0%.

Water <2.48> Not more than 0.2% (0.5 g, coulometric titration). For anolyte solution, use anode solution A for water determination.

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

Assay Weigh accurately about 50 mg each of Pioglitazone Hydrochloride and Pioglitazone Hydrochloride RS (separately, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), add exactly 10 mL of the internal standard solution and methanol to make 100 mL. Pipet 2 mL each of these solutions, add the mobile phase to make 20 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 pioglitazone to that of the internal standard.

$$\text{Amount (mg) of pioglitazone hydrochloride}$$
$$(C_{19}H_{20}N_2O_3S.HCl)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of benzophenone in methanol (1 in 750).
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 25°C.

Mobile phase: A mixture of ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).

Flow rate: Adjust so that the retention time of pioglitazone 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, pioglitazone 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Pioglitazone Hydrochloride Tablets

ピオグリタゾン塩酸塩錠

Pioglitazone Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$: 392.90).

Method of preparation Prepare as directed under Tablets, with Pioglitazone Hydrochloride.

Identification To an amount of powdered Pioglitazone Hydrochloride Tablets, equivalent to 2.8 mg of Pioglitazone Hydrochloride, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 267 nm and 271 nm.

Uniformity of dosage units <6.02> Perform the Mass variation test, or the Content uniformity test according to the fol-

lowing method: it meets the requirement.

Disintegrate 1 tablet of Pioglitazone Hydrochloride Tablets with 10 mL of 0.1 mol/L hydrochloric acid TS, add 70 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and centrifuge. Take exactly V mL of the supernatant liquid, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly V' mL so that each mL contains about 26 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Pioglitazone Hydrochloride RS (separately, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) 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 269 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a mixture of methanol and 0.1 mol/L hydrochloric acid TS (9:1) as the blank.

$$\text{Amount (mg) of pioglitazone hydrochloride}$$
$$(C_{19}H_{20}N_2O_3S.HCl)$$
$$= M_S \times A_T/A_S \times V'/V \times 2/25$$

M_S: Amount (mg) of Pioglitazone 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 a solution, which is prepared by mixing 50 mL of 0.2 mol/L hydrochloric acid TS and 150 mL of potassium chloride solution (3 in 20), adding water to make 1000 mL and adjusting to pH 2.0 with 5 mol/L hydrochloric acid TS, as the dissolution medium, the dissolution rate in 45 minutes of Pioglitazone Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride Tablets, withdraw 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 18 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pioglitazone Hydrochloride RS (separately determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in 10 mL of methanol, and add the dissolution medium to make exactly 50 mL. Pipet 2 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, of the sample solution and standard solution at 269 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using the dissolution medium as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount}$$
$$\text{of pioglitazone hydrochloride } (C_{19}H_{20}N_2O_3S.HCl)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 72$$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis
C Labeled amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$) in 1 tablet

Assay   Accurately weigh the mass of not less than 20 Pioglitazone Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), add 45 mL of methanol and exactly 5 mL of the internal standard solution, agitate with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Pioglitazone Hydrochloride RS (separately, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in 45 mL of methanol, and add exactly 5 mL of the internal standard solution. Pipet 2 mL of this solution, add the mobile phase 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 pioglitazone to that of the internal standard.

$$\text{Amount (mg) of pioglitazone hydrochloride}$$
$$(C_{19}H_{20}N_2O_3S.HCl)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of benzophenone in methanol (1 in 750).
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 25°C.
Mobile phase: A mixture of ammonium acetate solution (77 in 10,000), acetonitrile and acetic acid (100) (25:25:1).
Flow rate: Adjust so that the retention time of pioglitazone 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, pioglitazone 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pioglitazone to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Pioglitazone Hydrochloride and Glimepiride Tablets

ピオグリタゾン塩酸塩・グリメピリド錠

Pioglitazone Hydrochloride and Glimepiride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$: 392.90), and 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 Pioglitazone Hydrochloride and Glimepiride.

Identification   (1) Powder Pioglitazone Hydrochloride and Glimepiride Tablets, weigh a portion of the powder,

equivalent to 33 mg of Pioglitazone Hydrochloride, add 20 mL of 0.1 mol/L hydrochloric acid TS, and disintegrate completely by vigorous shaking for several minutes. Filter 2 mL of this solution through a membrane filter with a pore size not exceeding 0.45 μm. To 1 mL of the filtrate 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 267 nm and 271 nm.

(2) Wash the membrane filter obtained in (1) with 100 mL of 0.1 mol/L hydrochloric acid TS, and extract with methanol so that each mL contains 0.1 mg of glimepiride ($C_{24}H_{34}N_4O_5S$). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 227 nm and 231 nm.

Purity Related substances—Powder Pioglitazone Hydrochloride and Glimepiride Tablets, weigh a portion of the powder, equivalent to 10 mg of Glimepiride, add 30 mL of a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1), shake vigorously for 20 minutes, and add the mobile phase A to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.2 μm, discard the first 4 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 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 0.23 to glimepiride obtained from the sample solution is not larger than 2.5 times the peak area of glimepiride obtained from the standard solution. The area of the peak other than glimepiride and other than the peak mentioned above from the sample solution is not larger than 1/2 times the peak area of glimepiride from the standard solution, and the total area of these peaks is not larger than the peak area of glimepiride from the standard solution. 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: An ultraviolet absorption photometer (wavelength: 228 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: Dissolve 1.1 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 1.6 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 600 mL of acetonitrile.

Mobile phase B: Dissolve 1.1 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 1.6 with diluted phosphoric acid (1 in 10). To 300 mL of this solution add 700 mL of 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 – 15	100	0
15 – 60	100 → 0	0 → 100

Flow rate: 1.0 mL per minute.

Time span of measurement: For 60 minutes after injection, beginning after the peak having a relative retention time of about 0.23 to glimepiride.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase A to make exactly 20 mL. Confirm that the peak area of glimepiride obtained with 40 μL of this solution is equivalent to 7 to 13% of that obtained with 40 μL of the standard solution.

System performance: When the procedure is run with 40 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 20,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 40 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.

(1) Pioglitazone hydrochloride—To 1 tablet of Pioglitazone Hydrochloride and Glimepiride Tablets add 30 mL of a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1), shake vigorously for 20 minutes, and add the mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly $V'/10$ mL of the internal standard solution, add the mobile phase to make V' mL so that each mL contains about 66 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), and use this solution as the sample solution. Then, proceed as directed in the Assay (1).

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$)
  $= M_S \times Q_T/Q_S \times V'/V \times 1/10$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of ethyl benzoate in the mobile phase (1 in 10,000).

(2) Glimepiride—To 1 tablet of Pioglitazone Hydrochloride and Glimepiride Tablets add 30 mL of a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1), shake vigorously for 20 minutes, and add the mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly $V'/10$ mL of the internal standard solution, add the mobile phase to make V' mL so that each mL contains about 6 μg of glimepiride ($C_{24}H_{34}N_4O_5S$), and use this solution as the sample solution. Then, proceed as directed in the Assay (2).

Amount (mg) of glimepiride ($C_{24}H_{34}N_4O_5S$)
  $= M_S \times Q_T/Q_S \times V'/V \times 1/100$

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

Internal standard solution—A solution of ethyl benzoate in the mobile phase (1 in 10,000).

Dissolution <6.10> **(1)** Pioglitazone hydrochloride—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution, which is prepared by mixing 50 mL of 0.2 mol/L hydrochloric acid TS and 150 mL of potassium chloride solution (3 in 20), adding water to make 1000 mL and adjusting to pH 2.0 with 5 mol/L hydrochloric acid TS, as the dissolution medium, the dissolution rate in 45 minutes of Pioglitazone Hydrochloride and Glimepiride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride and Glimepiride Tablets, 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 18 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 37 mg of Pioglitazone Hydrochloride RS (separately determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in 20 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. 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 pioglitazone in each solution.

> Dissolution rate (%) with respect to the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl)
> $= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay (1) (Pioglitazone 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 pioglitazone are not less than 1500 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 conditions, the relative standard deviation of the peak area of pioglitazone is not more than 1.0 %.
(2) Glimepiride—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citrate buffer solution (pH 7.5) as the dissolution medium, the dissolution rate in 30 minutes of Pioglitazone Hydrochloride and Glimepiride Tablets is not less than 80%.
Start the test with 1 tablet of Pioglitazone Hydrochloride and Glimepiride Tablets, 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,

pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 1.1 μg of glimepiride ($C_{24}H_{34}N_4O_5S$), and use this solution as the sample solution. Separately, weigh accurately about 55 mg of Glimepiride RS (separately determine the water <2.48> in the same manner as Glimepiride), dissolve in acetonitrile to make exactly 250 mL. Pipet 10 mL of this solution, and add acetonitrile 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. 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.

> Dissolution rate (%) with respect to the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$)
> $= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/5$

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, column temperature and mobile phase: Proceed as directed in the operating conditions in the Assay (1) (Pioglitazone Hydrochloride).
Flow rate: Adjust so that the retention time of glimepiride is about 5.4 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 glimepiride 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 conditions, the relative standard deviation of the peak area of glimepiride is not more than 2.0%.

Assay **(1)** Pioglitazone hydrochloride—Weigh accurately the mass of not less than 20 Pioglitazone Hydrochloride and Glimepiride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 33 mg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl), add 30 mL of a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1), shake vigorously for 20 minutes, and add a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 5 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 33 mg of Pioglitazone Hydrochloride RS (separately determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), dissolve in a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) 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 pioglitazone to that of the internal standard.

Amount (mg) of pioglitazone hydrochloride
$$(\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_3\text{S}.\text{HCl})$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl benzoate in the mobile phase (1 in 10,000).
Operating conditions—
　Detector: An ultraviolet absorption photometer (wavelength: 228 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 25°C.
　Mobile phase: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 500 mL of this solution add 500 mL of acetonitrile.
　Flow rate: Adjust so that the retention time of pioglitazone is about 2.3 minutes.
System suitability—
　System performance: To 33 mg of Pioglitazone Hydrochloride RS add 5 mL of the glimepiride standard stock solution obtained in (2), and add a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) to make 50 mL. To 5 mL of this solution add 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, pioglitazone, the internal standard, and glimepiride are eluted in this order, and the resolutions between the peaks of pioglitazone and the internal standard and between the peaks of the internal standard and glimepiride are not less than 4 and not less than 3, respectively.
　System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of pioglitazone to that of the internal standard is not more than 1.0%.

　(2)　Glimepiride—Weigh accurately the mass of not less than 20 Pioglitazone Hydrochloride and Glimepiride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of glimepiride ($\text{C}_{24}\text{H}_{34}\text{N}_4\text{O}_5\text{S}$), add 30 mL of a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1), shake vigorously for 20 minutes, and add a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.2 μm. Discard the first 5 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 30 mg of Glimepiride RS (separately determine the water <2.48> in the same manner as Glimepiride), dissolve in the mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) to make exactly 50 mL, and use this solution as the glimepiride standard stock solution. Pipet 10 mL of the glimepiride standard stock solution, and add the mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) 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 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 glimepiride to that of the internal standard.

Amount (mg) of glimepiride ($\text{C}_{24}\text{H}_{34}\text{N}_4\text{O}_5\text{S}$)
$$= M_S \times Q_T/Q_S \times 1/10$$

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

Internal standard solution—A solution of ethyl benzoate in the mobile phase (1 in 10,000).
Operating conditions—
　Proceed as directed in the operating conditions in the Assay (1).
System suitability—
　System performance: To 33 mg of Pioglitazone Hydrochloride RS add 5 mL of the glimepiride standard stock solution, and add a mixture of acetonitrile and 0.1 mol/L hydrochloric acid TS (9:1) to make 50 mL. To 5 mL of this solution add 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, pioglitazone, the internal standard and glimepiride are eluted in this order, and the resolutions between the peaks of pioglitazone and the internal standard and between the peaks of the internal standard and glimepiride are not less than 4 and not less than 3, respectively.
　System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above 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.

Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets

ピオグリタゾン塩酸塩・メトホルミン塩酸塩錠

　Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pioglitazone hydrochloride ($\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_3\text{S}.\text{HCl}$: 392.90) and metformin hydrochloride ($\text{C}_4\text{H}_{11}\text{N}_5.\text{HCl}$: 165.62).

Method of preparation　Prepare as directed under Tablets, with Pioglitazone Hydrochloride and Metformin Hydrochloride.

Identification　(1)　Shake vigorously a quantity of powdered Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, equivalent to 0.33 mg of Pioglitazone Hydrochloride, with 10 mL of water, and filter through a membrane filter with a pore size not exceeding 0.45 μm. After washing the membrane filter with 10 mL of water, dissolve the retained substance on the filter by running through 10 mL of 0.1 mol/L hydrochloric acid TS, and determine the absorption spectrum of the filtrate so obtained as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 267 nm and 271 nm.
　(2)　Shake vigorously a quantity of powdered Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, equivalent to 20 mg of Metformin Hydrochloride, with 50 mL of water, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 1 mL of the filtrate add 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

230 nm and 234 nm.

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

(1) Pioglitazone hydrochloride—To 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. To this solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, 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 exactly $V'/20$ mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make V' mL so that each mL contains about 16.5 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), and use this solution as the sample solution. Then, proceed as directed in the Assay (1).

Amount (mg) of pioglitazone hydrochloride
($C_{19}H_{20}N_2O_3S.HCl$)
$$= M_S \times Q_T/Q_S \times V'/V \times 1/20$$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2500).

(2) Metformin hydrochloride—To 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. To this solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, 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 exactly $V'/20$ mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make V' mL so that each mL contains about 0.25 mg of metformin hydrochloride ($C_4H_{11}N_5.HCl$), and use this solution as the sample solution. Then, proceed as directed in the Assay (2).

Amount (mg) of metformin hydrochloride ($C_4H_{11}N_5.HCl$)
$$= M_S \times Q_T/Q_S \times V'/V \times 1/2$$

M_S: Amount (mg) of metformin hydrochloride for assay taken

Internal standard solution—A solution of 4′-methoxyacetophenone in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2000).

Dissolution <6.10> (1) Pioglitazone hydrochloride—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution, which is prepared by mixing 50 mL of 0.2 mol/L hydrochloric acid TS and 150 mL of potassium chloride solution (3 in 20), adding water to make 1000 mL and adjusting to pH 2.0 with 5 mol/L hydrochloric acid TS, as the dissolution medium, the dissolution rate in 30 minutes of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, 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, pipet V mL of the subsequent filtrate, add the

dissolution medium to make exactly V' mL so that each mL contains about 18.4 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 37 mg of Pioglitazone Hydrochloride RS (separately, determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) 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. 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 pioglitazone in each solution.

Dissolution rate (%) with respect to the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay (1).
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 pioglitazone are not less than 8000 and not more than 2.0, 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 pioglitazone is not more than 1.0%.

(2) Metformin hydrochloride—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium used in (1), the dissolution rate in 30 minutes of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, 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, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 0.56 mg of metformin hydrochloride ($C_4H_{11}N_5.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 50 mL, 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 metformin in each solution.

Dissolution rate (%) with respect to the labeled amount of metformin hydrochloride ($C_4H_{11}N_5.HCl$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 1800$$

M_S: Amount (mg) of metformin hydrochloride for assay taken
C: Labeled amount (mg) of metformin hydrochloride

($C_4H_{11}N_5$.HCl) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay (2).

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 metformin are not less than 6000 and not more than 2.5, 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 metformin is not more than 1.0%.

Assay (1) Pioglitazone hydrochloride—Weigh accurately the mass of not less than 20 Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 33 mg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl), add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. Add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Pioglitazone Hydrochloride RS (separately determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) 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 pioglitazone to that of the internal standard.

$$\text{Amount (mg) of pioglitazone hydrochloride}$$
$$(C_{19}H_{20}N_2O_3S.HCl)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl para-hydroxybenzoate in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Column: A stainless steel column 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 25°C.
Mobile phase: Dissolve 7.2 g of sodium lauryl sulfate in 1000 mL of a mixture of a solution of ammonium dihydrogen phosphate (23 in 4000) and acetonitrile (1:1).
Flow rate: Adjust so that the retention time of pioglitazone 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, pioglitazone 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pioglitazone to that of the internal standard is not more than 1.0%.

(2) Metformin hydrochloride—Weigh accurately the mass of not less than 20 Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 g of metformin hydrochloride ($C_4H_{11}N_5$.HCl), add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. Add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 10 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) 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 metformin to that of the internal standard.

$$\text{Amount (mg) of metformin hydrochloride } (C_4H_{11}N_5.HCl)$$
$$= M_S \times Q_T/Q_S \times 10$$

M_S: Amount (mg) of metformin hydrochloride for assay taken

Internal standard solution—A solution of 4'-methoxyacetophenone in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 255 nm).
Column: A stainless steel column 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 25°C.
Mobile phase: Dissolve 7.2 g of sodium lauryl sulfate in 1000 mL of a mixture of a solution of ammonium dihydrogen phosphate (23 in 4000) and acetonitrile (1:1).
Flow rate: Adjust so that the retention time of metformin 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, metformin and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.
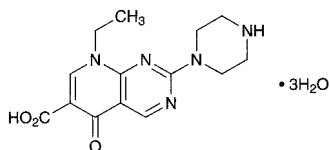
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the ratio of the peak area of metformin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pipemidic Acid Hydrate

ピペミド酸水和物

$C_{14}H_{17}N_5O_3.3H_2O$: 357.36
8-Ethyl-5-oxo-2-(piperazin-1-yl)-
5,8-dihydropyrido[2,3-*d*]pyrimidine-
6-carboxylic acid trihydrate
[*51940-44-4*, anhydride]

Pipemidic Acid Hydrate contains not less than 98.5% and not more than 101.0% of pipemidic acid ($C_{14}H_{17}N_5O_3$: 303.32), calculated on the anhydrous basis.

Description Pipemidic Acid Hydrate occurs as a pale yellow, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water and in ethanol (99.5), and practically insoluble in methanol.

It dissolves in sodium hydroxide TS.

It is gradually colored on exposure to light.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 0.1 g of Pipemidic Acid Hydrate in 20 mL of sodium hydroxide TS, and dilute with water to make 200 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.

(2) Determine the infrared absorption spectrum of Pipemidic Acid 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) Chloride <1.03>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute nitric acid, shake well, and filter through a glass filter (G3). 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 as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, then add 15 mL of dilute hydrochloric acid, shake well, and filter through a glass filter (G3). 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 5 mL of sodi-

um hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Pipemidic 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 10 ppm).

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

(5) Related substances—Dissolve 0.10 g of Pipemidic Acid Hydrate in 10 mL of diluted acetic acid (100) (1 in 20), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetic acid (100) (1 in 20) 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 chloroform, methanol, formic acid and triethylamine (25:15:5:1) 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> 14.5 – 16.0% (20 mg, coulometric titration).

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

Assay Weigh accurately about 0.35 g of Pipemidic Acid Hydrate, 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.
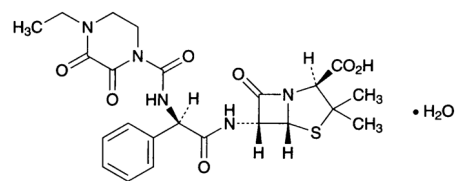
Each mL of 0.1 mol/L perchloric acid VS
= 30.33 mg of $C_{14}H_{17}N_5O_3$

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Piperacillin Hydrate

ピペラシリン水和物

$C_{23}H_{27}N_5O_7S.H_2O$: 535.57
(2*S*,5*R*,6*R*)-6-{(2*R*)-2-[(4-Ethyl-2,3-dioxopiperazine-
1-carbonyl)amino]-2-phenylacetylamino}-3,3-dimethyl-
7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
monohydrate
[*66258-76-2*]

Piperacillin Hydrate contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Hydrate is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Description Piperacillin Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5)

and in dimethylsulfoxide, and very slightly soluble in water.

Identification **(1)** Determine the infrared absorption spectrum of Piperacillin 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 Piperacillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the ¹H spectrum of a solution of Piperacillin Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 3) as directed under Nuclear Magnetic Resonance Spectroscopy *<2.21>*, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triple signal A at about δ 1.1 ppm, a single signal B at about δ 4.2 ppm, and a multiple signal C at about δ 7.4 ppm, and the ratio of the integrated intensity of each signal, A:B:C, is about 3:1:5.

Optical rotation *<2.49>* $[\alpha]_D^{20}$: +162 – +172° (0.2 g, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals *<1.07>*—Proceed with 2.0 g of Piperacillin 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 1—Conduct this procedure rapidly after the preparation of the sample solution and standard solution. Dissolve 20 mg of Piperacillin Hydrate 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 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and the standard solutions (1) and (2) 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, having the relative retention time of about 0.38 and about 0.50 to piperacillin, obtained from the sample solution is not larger than 2 times the peak area of piperacillin obtained from the standard solution (2), the total area of the peaks, having the relative retention time of about 0.82 and about 0.86 to piperacillin, from the sample solution is not larger than the peak area of piperacillin from the standard solution (2), and the area of the peak other than piperacillin and the peaks having the relative retention time of about 0.38, about 0.50, about 0.82 and about 0.86 to piperacillin, from the sample solution, is not larger than the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than piperacillin from the sample solution is not larger than the peak area of piperacillin from the standard solution (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 3 times as long as the retention time of piperacillin, beginning after the solvent peak.
System suitability—
Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that obtained from 20 μL of the standard solution (1).
System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin 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 (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 3.0%.

(3) Related substances 2—Dissolve 20 mg of Piperacillin Hydrate 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 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution, and the standard solutions (1) and (2) 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 6.6 to piperacillin, obtained from the sample solution is not larger than 3 times the peak area of piperacillin obtained from the standard solution (2), and the area of the peaks other than piperacillin and the peak mentioned above from the sample solution are not larger than 1.4 times the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than the peak of piperacillin from the sample solution is not larger than the area of the peak of piperacillin from the standard solution (1). For the area of the peak, having the relative retention time of about 6.6 to piperacillin, multiply the relative response factor, 2.0.
Operating conditions—
Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.
Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 300 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.
Flow rate: Adjust so that the retention time of piperacillin is about 1.2 minutes.
Time span of measurement: About 8 times as long as the retention time of piperacillin, beginning after the piperacillin peak.
System suitability—
Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that obtained from 20 μL of the standard solution (1).
System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 1500 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 4.0%.

(4) Residual solvents *<2.46>*—Transfer exactly 10 mg of Piperacillin Hydrate to an about 3 mL-vial, add exactly 1 mL of saturated sodium hydrogen carbonate solution to dissolve and stop the vial tightly. After heating this at 90°C for 10 minutes, use the gas inside the container as the sample gas. Separately, measure exactly 1 mL of ethyl acetate, dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 20 mL. Pipet 2 μL of this solution in an about 3-mL vial containing exactly 1 mL of saturated sodium hydrogen carbonate solution, and stop the vial tightly. Run the procedure similarly to the sample,

and use the gas as the standard gas. Perform the test with exactly 0.5 mL each of the sample gas and standard gas as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area of ethyl acetate by the automatic integration method: the peak area of ethyl acetate obtained from the sample gas is not larger than that obtained from the standard gas.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with porous stylene-divinyl benzene copolymer for gas chromatography (average pore diameter of $0.0085 \mu m$, $300 – 400 \ m^2/g$) with the particle size of 125 to $150 \mu m$.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethyl acetate is about 4 minutes.

System suitability—

System performance: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 μL each of ethyl acetate solution (1 in 400) and acetone solution (1 in 400), and stop the vial tightly. When the procedure is run under the above operating conditions, acetone and ethyl acetate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 μL of ethyl acetate solution (1 in 400), stop the vial tightly, and perform the test under the above operating conditions. When the procedure is repeated 6 times, the relative standard deviation of the peak area of ethyl acetate is not more than 10%.

Water <2.48>   Not less than 3.2% and not more than 3.8% (0.5 g, volumetric titration, direct titration).

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

Bacterial endotoxins <4.01>   Less than 0.07 EU/mg (potency).

Assay   Weigh accurately an amount of Piperacillin Hydrate and Piperacillin RS, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, 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, H_T and H_S, of the peak height of piperacillin to that of the internal standard.

Amount [μg (potency)] of piperacillin ($C_{23}H_{27}N_5O_7S$)
　　$= M_S \times H_T/H_S \times 1000$

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

Internal standard solution—A solution of acetanilide in the mobile phase (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 25°C.

Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 210 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of piperacillin 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, the internal standard and piperacillin 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Piperacillin Sodium

ピペラシリンナトリウム
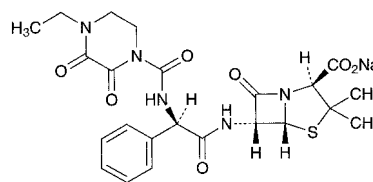
$C_{23}H_{26}N_5NaO_7S$: 539.54
Monosodium (2S,5R,6R)-6-{(2R)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenylacetylamino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
[*59703-84-3*]

Piperacillin Sodium contains not less than 863 μg (potency) and not more than 978 μg (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Sodium is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Description   Piperacillin Sodium occurs as a white powder or mass.

It is very soluble in water, freely soluble in methanol and in ethanol (95), and practically insoluble in acetonitrile.

Identification   (1)   Determine the infrared absorption spectrum of Piperacillin 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)   Piperacillin Sodium responds to Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+175 – +190°$ (0.8 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54>   Dissolve 1.0 g of Piperacillin Sodium in 4 mL of water: the pH of the solution is between 5.0 and 7.0.

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Piperacillin Sodium in 10 mL of water: the solution is clear and colorless.

(2)   Heavy metals <1.07>—Proceed with 2.0 g of Piperacillin Sodium 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) Arsenic *<1.11>*—Prepare the test solution with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.10 g of Piperacillin Sodium in 50 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A 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 areas of each peak by the automatic integration method: the area of the peak of ampicillin appeared at the retention time of about 7 minutes from the sample solution is not larger than 1/2 times that of piperacillin from the standard solution, the total area of related compounds 1 appeared at the retention times of about 17 minutes and about 21 minutes is not larger than 2 times of the peak area of piperacillin from the standard solution, the peak area of related compound 2 appeared at the retention time of about 56 minutes is not larger than that of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times of the peak area of piperacillin from the standard solution. For the peak areas of ampicillin, related compound 1 and related compound 2, multiply their relative response factors, 1.39, 1.32 and 1.11, 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 25°C.

Mobile phase A: A mixture of water, acetonitrile and 0.2 mol/L potassium dihydrogen phosphate (45:4:1).

Mobile phase B: A mixture of acetonitrile, water and 0.2 mol/L potassium dihydrogen phosphate (25:24:1).

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 – 7	100	0
7 – 13	100 → 83	0 → 17
13 – 41	83	17
41 – 56	83 → 20	17 → 80
56 – 60	20	80

Flow rate: 1.0 mL per minute (the retention time of piperacillin is about 33 minutes).

Time span of measurement: About 1.8 times as long as the retention time of piperacillin, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase A to make exactly 20 mL. Confirm that the peak area of piperacillin obtained from 20 μL of this solution is equivalent to 7 to 13% of that of piperacillin 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 piperacillin are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 2.0%.

Water *<2.48>* Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Piperacillin Sodium, equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Piperacillin RS, equivalent to about 0.1 g (potency), and dissolve in the mobile phase 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 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 height of piperacillin to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of piperacillin } (C_{23}H_{27}N_5O_7S)$$
$$= M_S \times Q_T/Q_S \times 1000$$

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

Internal standard solution—A solution of acetanilide in the mobile phase (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 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 60.1 g of acetic acid (100) and 101.0 g of triethylamine add water to make exactly 1000 mL. To 25 mL of this solution add 25 mL of dilute acetic acid and 210 mL of acetonitrile, and add water to make exactly 1000 mL.

Flow rate: Adjust so that the retention time of piperacillin 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, the internal standard and piperacillin 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Piperacillin Sodium for Injection

注射用ピペラシリンナトリウム

Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Method of preparation Prepare as directed under Injections, with Piperacillin Sodium.

Description Piperacillin Sodium for Injection is a white powder or masses.

Identification Proceed as directed in the Identification under Piperacillin Sodium.

pH *<2.54>* The pH of a solution prepared by dissolving an amount of Piperacillin Sodium for Injection, equivalent to 1.0 g (potency) of Piperacillin Sodium, in 4 mL of water is 5.0 – 7.0.

Purity (1) Clarity and color of solution—Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 4.0 g (potency) of Piperacillin Sodium, in 17 mL of water: the solution is clear and colorless.

(2) Related substances—Proceed as directed in the Purity (4) under Piperacillin Sodium.

Water *<2.48>* Not more than 1.0% (3 g, volumetric titration, direct titration).

Bacterial endotoxins *<4.01>* Less than 0.04 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 Piperacillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 20 mg (potency) of Piperacillin Sodium, dissolve in water to make exactly 20 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, weigh accurately about 20 mg (potency) of Piperacillin RS, and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Proceed as directed in the Assay under Piperacillin Sodium.

Amount [mg (potency)] of piperacillin ($C_{23}H_{27}N_5O_7S$)
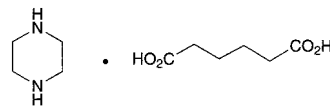= $M_S \times Q_T/Q_S$

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

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000).

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

Piperazine Adipate

ピペラジンアジピン酸塩

$C_4H_{10}N_2.C_6H_{10}O_4$: 232.28
Piperazine hexanedioate
[*142-88-1*]

Piperazine Adipate, when dried, contains not less than 98.5% of piperazine adipate ($C_4H_{10}N_2.C_6H_{10}O_4$).

Description Piperazine Adipate occurs as a white, crystalline powder. It is odorless, and has a slightly acid taste.

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

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 0.5 g of Piperazine Adipate in 10 mL of water, add 1 mL of hydrochloric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness on a water bath, and dry the residue at 105°C for 1 hour: the melting point *<2.60>* is between 152°C and 155°C.

(2) To 3 mL of a solution of Piperazine Adipate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Piperazine Adipate, 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 of 1.0 g of Piperazine Adipate in 20 mL of water is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Piperazine Adipate in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals *<1.07>*—Proceed with 2.0 g of Piperazine Adipate 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).

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 Piperazine Adipate, previously dried, dissolve in a mixture of 20 mL of acetic acid for nonaqueous titration and 40 mL of acetone for nonaqueous titration, and titrate *<2.50>* with 0.1 mol/L perchloric acid VS until the red-purple color of the solution changes to blue-purple (indicator: 6 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.61 mg of $C_4H_{10}N_2.C_6H_{10}O_4$

Containers and storage Containers—Well-closed containers.

Piperazine Phosphate Hydrate

ピペラジンリン酸塩水和物

$$\text{piperazine} \cdot H_3PO_4 \cdot H_2O$$
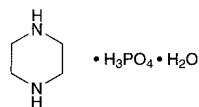
$C_4H_{10}N_2.H_3PO_4.H_2O$: 202.15
Piperazine monophosphate monohydrate
[*18534-18-4*]

Piperazine Phosphate Hydrate contains not less than 98.5% of piperazine phosphate ($C_4H_{10}N_2.H_3PO_4$: 184.13), calculated on the anhydrous basis.

Description   Piperazine Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

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

It dissolves in dilute hydrochloric acid.

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

Identification   **(1)**   To 3 mL of a solution of Piperazine Phosphate Hydrate (1 in 100) add 3 drops of Reinecke salt TS:a light red precipitate is formed.

(2)   Determine the infrared absorption spectrum of Piperazine Phosphate 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 Piperazine Phosphate Hydrate (1 in 100) responds to Qualitative Tests <*1.09*> (1) and (3) for phosphate.

pH <*2.54*>   Dissolve 1.0 g of Piperazine Phosphate Hydrate in 100 mL of water: the pH of the solution is between 6.0 and 6.5.

Purity   **(1)**   Chloride <*1.03*>—To 0.5 g of Piperazine Phosphate Hydrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(2)   Heavy metals <*1.07*>—To 2.0 g of Piperazine Phosphate Hydrate add 5 mL of dilute hydrochloric acid, 30 mL of water and 2 mL of dilute acetic acid, and dissolve. Add sodium hydroxide TS, adjust the pH of the solution to 3.3, and add water to make 50 mL. 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).

(3)   Arsenic <*1.11*>—Dissolve 2.0 g of Piperazine Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and use this solution as the test solution. Perform the test (not more than 1 ppm).

(4)   Related substances—Dissolve 50 mg of Piperazine Phosphate 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 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 cellulose for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28), acetone and ethanol (99.5) (8:3:3:2) to a distance of about 13 cm, and air-dry the plate. Spray evenly 4-dimethylaminocinnamaldehyde TS, and allow to stand for 15 minutes: the spots other than the principal spot and the spot on the starting line from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*>   8.0 – 9.5% (0.3 g, volumetric titration, direct titration).

Assay   Weigh accurately about 0.15 g of Piperazine Phosphate Hydrate, dissolve in 10 mL of formic acid, add 60 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
= 9.207 mg of $C_4H_{10}N_2.H_3PO_4$

Containers and storage   Containers—Well-closed containers.

Piperazine Phosphate Tablets

ピペラジンリン酸塩錠

Piperazine Phosphate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of piperazine phosphate hydrate ($C_4H_{10}N_2.H_3PO_4.H_2O$: 202.15).

Method of preparation   Prepare as directed under Tablets, with Piperazine Phosphate Hydrate.

Identification   Take a quantity of Piperazine Phosphate Tablets equivalent to 0.1 g of Piperazine Phosphate Hydrate, previously powdered, add 10 mL of water, shake while warming for 10 minutes, allow to cool, and filter. To 3 mL of the filtrate add 3 drops of Reinecke salt TS: a light red precipitate is formed.

Disintegration <*6.09*>   It meets the requirement. The time limit of the test is 10 minutes.

Assay   Weigh accurately not less than 20 Piperazine Phosphate Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of piperazine phosphate hydrate ($C_4H_{10}N_2.H_3PO_4.H_2O$). Add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. To the residue add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Repeat twice the same procedure with 5 mL each of acetic acid (100), combine all the supernatant liquids, 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
= 10.11 mg of $C_4H_{10}N_2.H_3PO_4.H_2O$

Containers and storage   Containers—Tight containers.

Pirarubicin

ピラルビシン
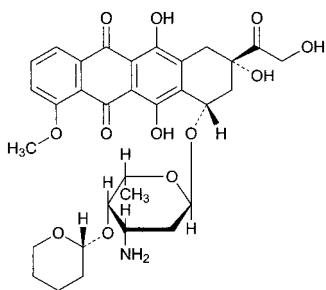
C$_{32}$H$_{37}$NO$_{12}$: 627.64
(2*S*,4*S*)-4-{3-Amino-2,3,6-trideoxy-4-*O*-[(2*R*)-3,4,5,6-tetrahydro-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyloxy}-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione
[*72496-41-4*]

Pirarubicin is a derivative of daunorubicin.

It contains not less than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pirarubicin is expressed as mass (potency) of pirarubicin (C$_{32}$H$_{37}$NO$_{12}$).

Description   Pirarubicin occurs as a red-orange crystalline powder.

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

Identification   (1)   Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) 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 or the spectrum of a solution of Pirarubicin 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 Pirarubicin and Pirarubicin RS in 5 mL of chloroform, 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 and methanol (5:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the necked eye: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a red-orange color and the same *R*f value.

Optical rotation <2.49>   [α]$_\mathrm{D}^{20}$: +195 – +215° (10 mg, chloroform, 10 mL, 100 mm).

Purity   (1)   Clarity and color of solution—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red.

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Pirarubicin 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 10 mg of Pirarubicin 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 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 doxorubicin, having the relative retention time of about 0.45 to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 to pirarubicin, obtained from the sample solution are not larger than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 to pirarubicin, from the sample solution is not larger than 5 times the peak area of pirarubicin from the standard solution. For the peak area for doxorubicin, multiply the relative response factor 0.94 and the area for the two peaks, having the relative retention times of about 1.9 and about 2.0, multiply their relative response factors, 1.09, 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 4 times as long as the retention time of pirarubicin.

System suitability—

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

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μL of this solution is equivalent to 14 to 26% of that obtained from 20 μL of the standard solution.

Water <2.48>   Not more than 2.0% (0.1 g, volumetric titration, direct titration).

Assay   Weigh accurately an amount of Pirarubicin and Pirarubicin RS, equivalent to about 10 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 5 mL of these solutions, add exactly 5 mL of the internal standard solution, 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 pirarubicin to that of the internal standard.

Amount [μg (potency)] of pirarubicin (C$_{32}$H$_{37}$NO$_{12}$)
   $= M_\mathrm{S} \times Q_\mathrm{T}/Q_\mathrm{S} \times 1000$

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

Internal standard solution—A solution of 2-naphthol in the mobile phase (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 0.05 mol/L ammonium formate buffer solution (pH 4.0) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of pirarubicin 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, pirarubicin 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pirarubicin to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Hermetic containers.

Pirenoxine

ピレノキシン
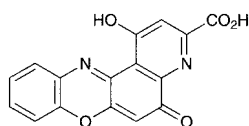
C$_{16}$H$_8$N$_2$O$_5$: 308.25
1-Hydroxy-5-oxo-5*H*-pyrido[3,2-*a*]phenoxazine-3-carboxylic acid
[*1043-21-6*]

Pirenoxine, when dried, contains not less than 98.0% of pirenoxine (C$_{16}$H$_8$N$_2$O$_5$).

Description   Pirenoxine occurs as a yellow-brown powder. It is odorless, and has a slightly bitter taste.

It is very slightly soluble in dimethylsulfoxide, and practically insoluble in water, in acetonitrile, in ethanol (95), in tetrahydrofuran and in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification   **(1)** Dissolve 2 mg of Pirenoxine in 10 mL of phosphate buffer solution (pH 6.5), add 5 mL of a solution of L-ascorbic acid (1 in 50), and shake vigorously: a dark purple precipitate is formed.

(2) Determine the absorption spectrum of a solution of Pirenoxine in phosphate buffer solution (pH 6.5) (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.

(3) Determine the infrared absorption spectrum of Pirenoxine, 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*>—Proceed with 1.0 g of Pirenoxine 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 Pirenoxine 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 200 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 total area of the peaks other than pirenoxine from the sample solution is not larger than the peak area of pirenoxine 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 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 1.39 g of tetra *n*-butylammonium chloride and 4.5 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust the pH to 6.5 with phosphoric acid. To 700 mL of this solution add 200 mL of acetonitrile and 30 mL of tetrahydrofuran, and mix.

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

Time span of measurement: About 3 times as long as the retention time of pirenoxine.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 30 mL. Confirm that the peak area of pirenoxine obtained from 5 μL of this solution is equivalent to 5 to 8% of that of pirenoxine obtained from 5 μL of the standard solution.

System performance: Dissolve 3 mg of Pirenoxine and 16 mg of methyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, pirenoxine and methyl 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 pirenoxine is not more than 1.0%.

Loss on drying <*2.41*>   Not more than 1.5% (0.5 g, in vacuum, 80°C, 3 hours).

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

Assay   Weigh accurately about 0.1 g of Pirenoxine, previously dried, dissolve in 140 mL of dimethylsulfoxide by heating on a water bath. After cooling, add 30 mL of water, and titrate <*2.50*> immediately with 0.02 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium hydroxide VS
= 6.165 mg of C$_{16}$H$_8$N$_2$O$_5$

Containers and storage   Containers—Tight containers.

Pirenzepine Hydrochloride Hydrate

ピレンゼピン塩酸塩水和物
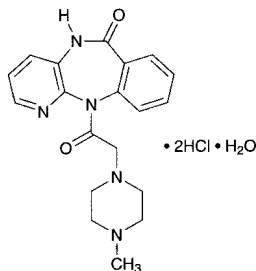
• 2HCl • H$_2$O

C$_{19}$H$_{21}$N$_5$O$_2$.2HCl.H$_2$O: 442.34
11-[(4-Methylpiperazin-1-yl)acetyl]-5,11-dihydro-6*H*-
pyrido[2,3-*b*][1,4]benzodiazepin-6-one dihydrochloride
monohydrate
[*29868-97-1*, anhydride]

Pirenzepine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of pirenzepine hydrochloride (C$_{19}$H$_{21}$N$_5$O$_2$.2HCl: 424.32), calculated on the anhydrous basis.

Description Pirenzepine Hydrochloride Hydrate occurs as a white to pale yellow crystalline powder.

It is freely soluble in water and in formic acid, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

The pH of a solution obtained by dissolving 1 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is between 1.0 and 2.0.

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

It is gradually colored by light.

Identification **(1)** Determine the absorption spectrum of a solution of Pirenzepine Hydrochloride Hydrate (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 Pirenzepine 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.

(3) A solution of Pirenzepine Hydrochloride Hydrate (1 in 50) responds to Qualitative Tests <1.09> for chloride.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is clear and not more color than that of the following control solution.

Control solution: To 1.2 mL of Matching Fluid F add 8.8 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pirenzepine 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).

(3) Related substances—Dissolve 0.3 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water. To 1 mL of this solution add 5 mL of methanol and the mobile phase A to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Pipet 1

mL of this solution, add 5 mL of methanol and the mobile phase A 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 area of the peak other than pirenzepine from the sample solution is not larger than 3/10 times the peak area of pirenzepine from the standard solution, and the total area of the peaks other than pirenzepine from the sample solution is not larger than 3/5 times the peak area of pirenzepine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 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: Dissolve 2 g of sodium lauryl sulfate in 900 mL of water, adjust the pH to 3.2 with acetic acid (100), and add water to make 1000 mL.

Mobile phase B: Methanol.

Mobile phase C: Acetonitrile.

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)
0 – 15	55 → 25	30	15 → 45
15 –	25	30	45

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

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Confirm that the peak area of pirenzepine 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 0.1 g of 1-phenylpiperazine hydrochloride in 10 mL of methanol. Mix 1 mL of this solution and 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pirenzepine and phenylpiperazine 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 area of pirenzepine is not more than 2.0%.

Water <2.48> Not less than 3.5% and 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 0.2 g of Pirenzepine Hydrochloride Hydrate, dissolve in 2 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 in the same manner, and make any necessary correction.
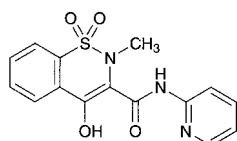
$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 14.14 \text{ mg of } C_{19}H_{21}N_5O_2 \cdot 2HCl$$

Containers and storage   Containers—Well-closed containers.

Storage—Light-resistant.

Piroxicam

ピロキシカム

C$_{15}$H$_{13}$N$_3$O$_4$S: 331.35
4-Hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide
[*36322-90-4*]

Piroxicam contains not less than 98.5% and not more than 101.0% of piroxicam (C$_{15}$H$_{13}$N$_3$O$_4$S), calculated on the dried basis.

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

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

Melting point: about 200°C (with decomposition).

It shows crystal polymorphism.

Identification   (1)   Dissolve 0.1 g of Piroxicam in a mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 200 mL. To 1 mL of this solution add the mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) 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 Piroxicam 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 dichloromethane, evaporate the solvent, dry the residue on a water bath, and perform the test.

Purity   (1)   Heavy metals <1.07>—Proceed with 1.0 g of Piroxicam 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 75 mg of Piroxicam in 50 mL of acetonitrile for liquid chromatography, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile for liquid chromatography to make exactly 10 mL. Pipet 1 mL of this solution, add acetonitrile for liquid chromatography 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 by the automatic integration method: the area of the peak other than piroxicam obtained with the sample solution is not larger than the peak area of piroxicam obtained with the standard solution, and the total area of the peaks other than piroxicam with the sample solution is not larger than 2 times the peak area of piroxicam with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0) and acetonitrile for liquid chromatography (3:2).

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

Time span of measurement: About 5 times as long as the retention time of piroxicam, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add acetonitrile for liquid chromatography to make exactly 20 mL. Confirm that the peak area of piroxicam obtained with 20 µL of this solution is equivalent to 17.5 to 32.5% 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 piroxicam are not less than 6000 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 piroxicam 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 0.2% (1 g).

Assay   Weigh accurately about 0.25 g of Piroxicam, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), 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.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 33.14 \text{ mg of } C_{15}H_{13}N_3O_4S$$

Containers and storage   Containers—Tight containers.

Pitavastatin Calcium Hydrate

ピタバスタチンカルシウム水和物
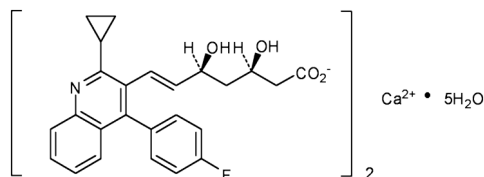
$C_{50}H_{46}CaF_2N_2O_8 \cdot 5H_2O$: 971.06
Monocalcium bis{(3R,5S,6E)-7-[2-cyclopropyl-
4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyhept-
6-enoate} pentahydrate
[147526-32-7, anhydride]

Pitavastatin Calcium Hydrate contains not less than 98.0% and not more than 102.0% of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$: 880.98), calculated on the anhydrous basis.

Description Pitavastatin Calcium Hydrate occurs as a white to pale yellow powder.

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

It dissolves in dilute hydrochloric acid.

It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Pitavastatin Calcium Hydrate in methanol (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.

(2) Determine the infrared absorption spectrum of Pitavastatin Calcium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of 3400 – 3300 cm⁻¹, about 1560 cm⁻¹, 1490 cm⁻¹, 1219 cm⁻¹, 1066 cm⁻¹ and 766 cm⁻¹.

(3) Dissolve 0.25 g of Pitavastatin Calcium Hydrate in 5 mL of dilute hydrochloric acid, neutralize with ammonia TS, and filter: the filtrate responds to the Qualitative Tests <1.09> (1), (2), and (3) for calcium.

Optical rotation <2.49> $[\alpha]_D^{20}$: +22.0 – +24.5° (0.1 g calculated on the anhydrous basis, a mixture of water and acetonitrile (1:1), 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—To 1.0 g of Pitavastatin Calcium Hydrate in a quartz crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and mix well, then fire the ethanol to burn, and heat gradually to carbonize. After cooling, moisten the residue with 1.5 mL of sulfuric acid, heat carefully, then ignite at 550°C until the residue is incinerated. After cooling, moisten the residue with 1.5 mL of nitric acid, heat carefully, then ignite at 550°C until the residue is completely incinerated. After cooling, dissolve the residue in 3 mL of hydrochloric acid, and evaporate the solvent to dryness on a water bath. Moisten the residue with 3 drops of hydrochloric acid, dissolve in 10 mL of hot water with the aid of gentle heat, and filter. Wash the residue with 20 mL of water, and pour the filtrates and washings into a Nessler tube. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, then add 2 mL of dilute acetic acid, add water to make 50 mL, and use this solution

as the test solution. The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. Hereafter, proceed as for the test solution, then add 2.0 mL of Standard Lead Solution, 2 mL of acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Pitavastatin Calcium Hydrate in 100 mL of a mixture of acetonitrile and water (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) 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 1.1 to pitavastatin, obtained from the sample solution is not more than 1/2 times the peak area of pitavastatin obtained from the standard solution, and the area of the peak other than pitavastatin and the peak, having the relative retention time of about 1.1, from the sample solution is not more than 1/10 times the peak area of pitavastatin from the standard solution. Furthermore, the total area of the peaks other than pitavastatin from the sample solution is not larger than the peak area of pitavastatin from the standard solution. For the area of the peak, having the relative retention time of about 1.4 to pitavastatin, multiply the relative response factor, 1.8.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: To 10 mL of dilute acetic acid add water to make 1000 mL. To 800 mL of this solution add diluted sodium acetate TS (1 in 100) to adjust to pH 3.8.

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	60	40
20 – 40	60 → 10	40 → 90
40 – 60	10	90

Flow rate: Adjust so that the retention time of pitavastatin is about 23 minutes.

Time span of measurement: About 2.5 times as long as the retention time of pitavastatin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of acetonitrile and water (3:2) to make exactly 20 mL. Confirm that the peak area of pitavastatin obtained with 10 μL of this solution is equivalent to 4 to 6% 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 pitavastatin are not less than 17,000 and not more than 1.3, 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 pitavastatin is not more than 2.0%.

Water <2.48> 9.0 – 13.0% (0.2 g, volumetric titration, direct titration. Use a mixture of pyridine for water determination and ethylene glycol for water determination (83:17) instead of methanol for water determination).

Assay Conduct this procedure using light-resistant vessels.

Weigh accurately about 0.1 g of Pitavastatin Calcium Hydrate, dissolve in a mixture of acetonitrile and water (3:2) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pitavastatin Methylbenzylamine RS (separately determine the water <2.48> by coulometric titration using 0.1 g), dissolve in a mixture of acetonitrile and water (3:2) to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) 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 pitavastatin to that of the internal standard.

Amount (mg) of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$)
$= M_S \times Q_T/Q_S \times 4 \times 0.812$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS taken, calculated on the anhydrous basis

Internal standard solution—Butyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (3 in 2000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 245 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: To 10 mL of dilute acetic acid add water to make 1000 mL. To 350 mL of this solution add 650 mL of methanol, and dissolve 0.29 g of sodium chloride in this solution.
Flow rate: Adjust so that the retention time of pitavastatin is about 17 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 pitavastatin 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 ratio of the peak area of pitavastatin to that of the internal standard is not more than 1.0%.

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

Pitavastatin Calcium Tablets

ピタバスタチンカルシウム錠

Pitavastatin Calcium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$: 880.98).

Method of preparation Prepare as directed under Tablets, with Pitavastatin Calcium Hydrate.

Identification Powder Pitavastatin Calcium Tablets. Weigh a portion of the powder, equivalent to 4 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), add 10 mL of methanol and shake well, and centrifuge. To 1 mL of the supernatant liquid, add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 242 nm and 246 nm.

Purity Related substances—Conduct this procedure using light-resistant vessels. Take a quantity of Pitavastatin Calcium Tablets, equivalent to 20 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), add 60 mL of a mixture of acetonitrile and water (3:2), and disintegrate the tablets with the aid of ultrasonic waves. To this dispersed solution, add a mixture of acetonitrile and water (3:2) to make 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Perform the test with 50 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. Calculate the amount of the peaks by the area percentage method: the amount of the peak, having the relative retention time of about 1.1 and about 1.7 to pitavastatin, obtained from sample solution is not more than 0.5%, the amount of the peak other than pitavastatin and the peaks mentioned above is not more than 0.1%, and the total amount of the peaks other than pitavastatin is not more than 1.5%.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 245 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: To 10 mL of dilute acetic acid add water to make 1000 mL. To 800 mL of this solution add diluted sodium acetate TS (1 in 100) to adjust to pH 3.8.
Mobile phases 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	60	40
20 – 40	60 → 30	40 → 70
40 – 65	30	70

Flow rate: Adjust so that the retention time of pitavastatin is about 23 minutes.
Time span of measurement: About 2.7 times as long as the

retention time of pitavastatin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of acetonitrile and water (3:2) 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 acetonitrile and water (3:2) to make exactly 50 mL. Confirm that the peak area of pitavastatin obtained with 50 μL of this solution is equivalent to 7 to 13% of that obtained with 50 μL of the solution for system suitability test.

System performance: When the procedure is run with 50 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 pitavastatin are not less than 7500 and not more than 2.0, respectively.

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 pitavastatin 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.

Conduct this procedure using light-resistant vessels. To 1 tablet of Pitavastatin Calcium Tablets add exactly V mL of the internal standard solution so that each mL contains about 0.2 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), and add V mL of a mixture of acetonitrile and water (3:2), shake well until the tablet is disintegrated completely. Filter this solution 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 pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$)
$$= M_S \times Q_T/Q_S \times V/100 \times 0.812$$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of butyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (3 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 rates in 15 minutes of Pitavastatin Calcium Tablets is not less than 85%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Pitavastatin Calcium Tablets, 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 not less than 5 mL of the first 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 pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), and use this solution as the sample solution. Separately, weigh accurately about 24 mg of Pitavastatin Methylbenzylamine RS (separately determine the water), and dissolve in a mixture of acetonitrile and water (3:2) to make exactly 200 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 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 pitavastatin in each solution.

Dissolution rate (%) with respect to the labeled amount of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2 \times 0.812$$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$) 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 pitavastatin are not less than 4500 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 pitavastatin is not more than 2.0%.

Assay   Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Pitavastatin Calcium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), add 30 mL of a mixture of acetonitrile and water (3:2), and treat with the ultrasonic waves for 10 minutes. To this solution, add a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Pipet 5 mL of this filtrate, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of Pitavastatin Methylbenzylamine RS (separately determine the water *<2.48>* by coulometric titration using 0.1 g), and dissolve in a mixture of acetonitrile and water (3:2) 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 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 pitavastatin to that of the internal standard.

Amount (mg) of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$)
$$= M_S \times Q_T/Q_S \times 1/2 \times 0.812$$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS taken, calculated on the anhydrous basis

*Internal standard solution—*A solution of butyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (3 in 10,000).

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 (3 μm in particle diameter).

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

Mobile phase: To 10 mL of dilute acetic acid add water to make 1000 mL. To 350 mL of this solution add 650 mL of methanol, and dissolve 0.29 g of sodium chloride in this solution.

Flow rate: Adjust so that the retention time of pitavastatin 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 internal standard and pitavastatin 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 pitavastatin to that of the internal standard is not more than 1.0%.

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

Pivmecillinam Hydrochloride

ピブメシリナム塩酸塩
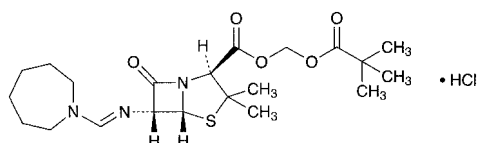
C$_{21}$H$_{33}$N$_3$O$_5$S.HCl: 476.03
2,2-Dimethylpropanoyloxymethyl (2*S*,5*R*,6*R*)-6-[(azepan-1-ylmethylene)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride
[*32887-03-9*]

Pivmecillinam Hydrochloride contains not less than 630 μg (potency) and not more than 710 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pivmecillinam Hydrochloride is expressed as mass (potency) of mecillinam (C$_{15}$H$_{23}$N$_3$O$_3$S: 325.43).

Description   Pivmecillinam Hydrochloride occurs as a white to yellowish white crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (99.5), and soluble in acetonitrile.

Identification   **(1)**   Determine the infrared absorption spectrum of Pivmecillinam 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 Pivmecillinam Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2)   Dissolve 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49>   [α]$_D^{20}$: +200 – +220° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Purity   **(1)**   Heavy metals <1.07>—To 1.0 g of Pivmecillinam Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 3 mL of hydrochloric acid to the residue, dissolve by warming on a water bath, and heat to dryness. To the residue add 10 mL of water, and dissolve by warming on a water bath. After cooling, adjust the pH to 3 to 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, and wash the crucible and the filter with 10 mL of water. Put the filtrate and the washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution in the same manner as the test 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 Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3)   Related substances—Dissolve 50 mg of Pivmecillinam Hydrochloride in 4.0 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the sample solution. Separately, dissolve 2.0 mg of Pivmecillinam Hydrochloride RS in 4.0 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 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, then spot 2 μL of the sample solution on the plate. Immediately, develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 10 minutes in iodine vapor: the spot obtained from the sample solution appeared at the position corresponding to the spot obtained from the standard solution is not larger and not more intense than the spot from the standard solution, and any spot other than the principal spot and the above spot from the sample solution is not observable.

Water <2.48>   Not more than 1.0% (0.25 g, coulometric titration).

Assay   Weigh accurately an amount of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase 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 pivmecillinam to that of the internal standard.

Amount [μg (potency)] of mecillinam (C$_{15}$H$_{23}$N$_3$O$_3$S)
    = $M_S \times Q_T/Q_S \times 1000$

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

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).
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: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust so that the retention time of pivmecillinam is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating con-

ditions, pivmecillinam 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 ratios of the peak area of pivmecillinam to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Pivmecillinam Hydrochloride Tablets

ピブメシリナム塩酸塩錠

Pivmecillinam Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled potency of mecillinam ($C_{15}H_{23}N_3O_3S$: 325.43).

Method of preparation   Prepare as directed under Tablets, with Pivmecillinam Hydrochloride.

Identification   Powder Pivmecillinam Hydrochloride Tablets, dissolve a portion of the powder, equivalent to 35 mg (potency) of Pivmecillinam Hydrochloride, in 4 mL of a mixture of acetonitrile and acetic acid (100) (97:3), 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 dissolve 25 mg of Pivmecillinam Hydrochloride RS in 2 mL of a mixture of acetonitrile and acetic acid (100) (97:3), 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, and immediately develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 10 minutes: the principal spot obtained from the sample solution has the same Rf value as the spot obtained from the standard solution.

Water <2.48>   Not more than 3.0% (1 g of powdered Pivmecillinam Hydrochloride Tablets, volumetric titration, direct titration).

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 Pivmecillinam Hydrochloride Tablets add 40 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 50 mL. Pipet V mL, equivalent to about 10 mg (potency) of Pivmecillinam Hydrochloride, add exactly 5 mL of the internal standard solution and the mobile phase 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. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

Amount [mg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$)
  $= M_S \times Q_T/Q_S \times 25/V$

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

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

Disintegration <6.09>   Perform the test using the disk: it meets the requiroement.

Assay   Weigh accurately the mass of not less than 20 Pivmecillinam Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Pivmecillinam Hydrochloride, add 50 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase 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. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride RS, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

Amount [mg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$)
  $= M_S \times Q_T/Q_S \times 5$

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

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

Containers and storage   Containers—Tight containers.

Live Oral Poliomyelitis Vaccine

経口生ポリオワクチン

Live Oral Poliomyelitis Vaccine contains live attenuated poliovirus of type I, II and III.

Monovalent or bivalent product may be prepared, if necessary.

Live Oral Poliomyelitis Vaccine conforms to the requirements of Live Oral Poliomyelitis Vaccine in the Minimum Requirements for Biological Products.

Description   Live Oral Poliomyelitis Vaccine is a light yellow-red to light red, clear liquid.

Polymixin B Sulfate

ポリミキシン B 硫酸塩

Polymixin B$_1$: R = CH$_3$ Dbu =

Polymixin B$_2$: R = H   Dbu =
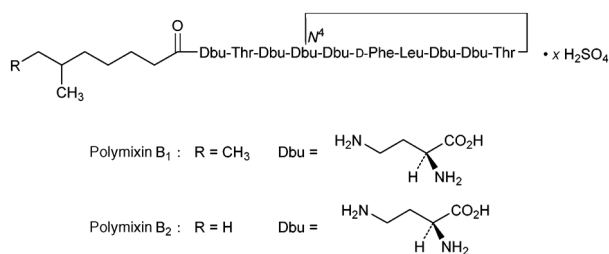
Polymixin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa*.

It contains not less than 6500 units per mg, calculated on the dried basis. The potency of Polymixin B Sulfate is expressed as mass unit of polymixin B (C$_{55\text{-}56}$H$_{96\text{-}98}$N$_{16}$O$_{13}$). One unit of Polymixin B Sulfate is equivalent to 0.129 μg of polymixin B sulfate (C$_{55\text{-}56}$H$_{96\text{-}98}$N$_{16}$O$_{13}$.1-2H$_2$SO$_4$).

Description   Polymixin B Sulfate occurs as a white to yellow-brown powder.

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

Identification   (1)   To 5 mL of a solution of Polymixin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), add 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

(2)   Transfer 5 mg each of Polymixin B Sulfate and Polymixin B Sulfate RS separately into two glass stoppered test tubes, add 1 mL of diluted hydrochloric acid (1 in 2), stopper the tube, heat at 135°C for 5 hours, then heat to dryness on a water bath, and keep the heating until no more hydrochloric acid odor is evolved. Dissolve the residue in 0.5 mL of water, and use these solutions as the sample solution and standard solution (1). Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine separately in 10 mL of water, and use these solutions as the standard solutions (2), (3), (4) and (5), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 3 μL each of the sample solution, the standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography, and expose the plate to a saturated vapor of the developing solvent for 15 hours. Develop the plate with a mixture of phenol and water (3:1) to a distance of about 13 cm while without exposure to light, and dry the plate at 110°C for 5 minutes. Spray evenly ninhydrin-acetic acid TS on the plate, and heat at 110°C for 5 minutes: *R*f value of each spot obtained from the sample solution is the same with *R*f value of the corresponding spots obtained from the standard solution (1). Each of the spots from the sample solution appears at the position corresponding to each of the spots from the standard solutions (2), (3) and (4), but not appears at the position corresponding to the spot from the standard solution (5).

(3)   A solution of Polymixin B Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

Optical rotation <2.49>   [α]$_\text{D}^{20}$: -78 – $-90°$ (0.5 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 Polymixin B Sulfate in 50 mL of water is between 5.0 and 7.0.

Phenylalanine   Weigh accurately about 0.375 g of Polymixin B Sulfate, dissolve in 0.1 mol/L hydrochloric acid VS to make exactly 100 mL. Determine absorbances, A_1, A_2, A_3, A_4 and A_5, of this solution at 252 nm, at 258 nm, at 264 nm, at 280 nm and at 300 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate the amount of phenylalanine by the following equation: the amount of phenylalanine is not less than 9.0% and not more than 12.0%.

Amount (%) of phenylalanine
$$= (A_2 - 0.5A_1 + 0.5A_3 - 1.8A_4 + 0.8A_5)/M_\text{T} \times 9.4787$$

M_T: Amount (g) of Polymixin B Sulfate taken, calculated on the dried basis

Purity   Heavy metals <1.07>—Proceed with 1.0 g of Polymixin B 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).

Loss on drying <2.41>   Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44>   Not more than 0.75% (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—*Escherichia coli* NIHJ
   (ii)   Agar media for seed and base layer

Peptone	10.0 g
Meat extract	3.0 g
Sodium chloride	30.0 g
Agar	20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH <2.54> of the solution so that it will be 6.5 to 6.6 after sterilization.

   (iii)   Standard solutions—Weigh accurately an amount of Polymixin B Sulfate RS, equivalent to about 200,000 units, dissolve in phosphate buffer solution (pH 6.0) to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. 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 4000 units and 1000 units, 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 Polymixin B Sulfate, equivalent to about 200,000 units, and dissolve in phosphate buffer solution (pH 6.0) to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 4000 units and 1000 units, 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.

Polyoxyl 40 Stearate

ステアリン酸ポリオキシル 40

Polyoxyl 40 Stearate is the monostearate of condensation polymers of ethylene oxide represented by the formula $H(OCH_2CH_2)_nOCOC_{17}H_{35}$, in which *n* is approximately 40.

Description   Polyoxyl 40 Stearate occurs as a white to light yellow, waxy solid or powder. It is odorless or has a faint fat-like odor.

It is soluble in water, in ethanol (95) and in diethyl ether.

Congealing point <*2.42*>   39.0 – 44.0°C

Congealing point of the fatty acid <*1.13*>   Not below 53°C.

Acid value <*1.13*>   Not more than 1.

Saponification value <*1.13*>   25 – 35

Purity   **(1)**   Clarity and color of solution—Dissolve 1.0 g of Polyoxyl 40 Stearate in 20 mL of water: the solution is clear and colorless.

(2)   Heavy metals <*1.07*>—Proceed with 2.0 g of Polyoxyl 40 Stearate 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 0.67 g of Polyoxyl 40 Stearate, according to Method 3, and perform the test (not more than 3 ppm).

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

Containers and storage   Containers—Tight containers.

Polysorbate 80

ポリソルベート 80

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 (♦   ♦).

Polysorbate 80 is a mixture of partial esters of fatty acids, mainly oleic acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

♦**Description**   Polysorbate 80 is a colorless or brownish yellow, clear or slightly opalescent, oily liquid.

It is miscible with water, with methanol, with ethanol (99.5) and with ethyl acetate.

It is practically insoluble in fatty oils and in liquid paraffin.

Viscosity: about 400 mPa·s (25°C).

Specific gravity d_{20}^{20}: about 1.10.♦

Identification   It meets the requirements of the Composition of fatty acids.

Composition of fatty acids   Dissolve 0.10 g of Polysorbate 80 in 2 mL of a solution of sodium hydroxide in methanol (1 in 50) in a 25-mL conical flask, and boil under a reflux condenser for 30 minutes. Add 2.0 mL of boron trifluoride-methanol TS through the condenser, and boil for 30 minutes. Add 4 mL of heptane through the condenser, and boil for 5 minutes. After cooling, add 10.0 mL of saturated sodium chloride solution, shake for about 15 seconds, and add a quantity of saturated sodium chloride solution such that the upper phase is brought into the neck of the flask. Collect 2 mL of the upper phase, wash with three 2-mL portions of water, dry with anhydrous sodium sulfate, and use this solution as the sample solution. Perform the test with 1 µL each of the sample solution and fatty acid methyl esters mixture TS as directed under Gas Chromatography <*2.02*> according to the following conditions. Identify each peaks obtained with the sample solution using the chromatogram obtained with fatty acid methyl esters mixture TS. Determine each peak area with the sample solution by the automatic integration method, and calculate the composition of fatty acids by the area percentage method: myristic acid is not more than 5.0%, palmitic acid is not more than 16.0%, palmitoleic acid is not more than 8.0%, stearic acid is not more than 6.0%, oleic acid is not less than 58.0%, linoleic acid is not more than 18.0% and linolenic acid is not more than 4.0%.

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 inside surface with polyethylene glycol 20 M for gas chromatography 0.5 µm in thickness.

Column temperature: Inject at a constant temperature of about 80°C, rise the temperature at the rate of 10°C per minute to 220°C, and maintain at 220°C for 40 minutes.

Injection port temperature: A constant temperature of about 250°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: 50 cm per second.

System suitability—

Test for required detectability: Dissolve 0.50 g of the mixture of fatty acid methyl esters described in the following table in heptane to make exactly 50 mL, and use this solution as the solution for system suitability test. To 1.0 mL of the solution for system suitability test add heptane to make 10.0 mL. When the procedure is run with 1 µL of this solution under the above operating conditions, the SN ratio of methyl myristate is not less than 5.

Mixture of fatty acid methyl esters	Composition (%)
Methyl myristate for gas chromatography	5
Methyl palmitate for gas chromatography	10
Methyl stearate for gas chromatography	15
Methyl arachidate for gas chromatography	20
Methyl oleate for gas chromatography	20
Methyl eicosenoate for gas chromatography	10
Methyl behenate	10
Methyl lignocerate for gas chromatography	10

System performance: When the procedure is run with 1 µL of the solution for system suitability test under the above operating conditions, ♦methyl stearate and methyl oleate are eluted in this order,♦ the resolution between these peaks is not less than 1.8, and the number of theoretical plates of the peak of methyl stearate is not less than 30,000.

♦**Acid value** <*1.13*>   Not more than 2.0 (using ethanol (95) instead).♦

Saponification value   Introduce about 4 g of Polysorbate 80

into a 250-mL borosilicate glass flask. Add exactly 30 mL of 0.5 mol/L potassium hydroxide-ethanol VS and a few glass beads. Attach a reflux condenser, and heat for 60 minutes. Add 1 mL of phenolphthalein TS and 50 mL of ethanol (99.5), and titrate ⟨2.50⟩ immediately with 0.5 mol/L hydrochloric acid VS. Perform a blank determination in the same manner. Calculate the saponification value by the following equation: 45 – 55.

$$\text{Saponification value} = (a - b) \times 28.05/M$$

M: Amount (g) of Polysorbate 80 taken
a: Volume (mL) of 0.5 mol/L hydrochloric acid VS required for blank determination
b: Volume (mL) of 0.5 mol/L hydrochloric acid VS required for sample determination

Hydroxyl value   Introduce about 2 g of Polysorbate 80 into a 150-mL round bottom flask, add exactly 5 mL of acetic anhydride-pyridine TS, and attach an air condenser. Heat the flask in a water bath for 1 hour keeping the level of the water about 2.5 cm above the level of the liquid in the flask. Withdraw the flask and allow to cool. Add 5 mL of water through the condenser. If a cloudiness appears add sufficient pyridine to clear it, noting the volume added. Shake the flask, and heat in the water bath for 10 minutes. Withdraw the flask and allow to cool. Rinse the condenser and the walls of the flask with 5 mL of neutralized ethanol, and titrate ⟨2.50⟩ with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 0.2 mL of phenolphthalein TS). Perform a blank determination in the same manner. Calculate the hydroxyl value by the following equation: 65 – 80.

$$\text{Hydroxyl value} = (a - b) \times 28.05/M + \text{acid value}$$

M: Amount (g) of Polysorbate 80 taken
a: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS required for blank determination
b: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS required for sample determination

Purity   ◆(1)   Heavy metals ⟨1.07⟩—Proceed with 1.0 g of Polysorbate 80 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)   Ethylene oxide and 1,4-dioxane—Transfer exactly 1.00 g of Polysorbate 80 into a 10-mL headspace vial, add exactly 2 mL of water, seal the vial immediately with a septum of silicon rubber coated the surface with fluororesin and an aluminum cap. Mix carefully, and use the content as the sample solution. Separately, pipet 0.5 mL of a solution, prepared by dissolving ethylene oxide in dichloromethane so that each mL contains 50 mg, and add water to make exactly 50 mL. Allow to stand to reach room temperature. Pipet 1 mL of this solution, add water to make exactly 250 mL, and use this solution as ethylene oxide stock solution. Separately, pipet 1 mL of 1,4-dioxane, add water to make exactly 200 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as 1,4-dioxane stock solution. To exact 6 mL of ethylene oxide stock solution and exact 2.5 mL of 1,4-dioxane stock solution add water to make exactly 25 mL, and use this solution as ethylene oxide-1,4-dioxane standard stock solution. Separately, transfer exactly 1.00 g of Polysorbate 80 into a 10-mL headspace vial, add exactly 2 mL of ethylene oxide-1,4-dioxane standard stock solution, seal the vial immediately with a septum of silicon rubber coated the surface with fluororesin and an aluminum cap. Mix carefully, and use the content as the standard solution. Perform the test with the sample solution and standard solution as directed in the head-space method under Gas

Chromatography ⟨2.02⟩ according to the following conditions. The amounts of ethylene oxide and 1,4-dioxane, calculated by the following equations, are not more than 1 ppm and not more than 10 ppm, respectively.

$$\text{Amount (ppm) of ethylene oxide} = 2 \times C_{EO} \times A_a/(A_b - A_a)$$

C_{EO}: Concentration (μg/mL) of added ethylene oxide in the standard solution
A_a: Peak area of ethylene oxide obtained with the sample solution
A_b: Peak area of ethylene oxide obtained with the standard solution

$$\text{Amount (ppm) of 1,4-dioxane} = 2 \times 1.03 \times C_D \times A'_a \times 1000/(A'_b - A'_a)$$

C_D: Concentration (μL/mL) of added 1,4-dioxane in the standard solution
1.03: Density (g/mL) of 1,4-dioxane
A'_a: Peak area of 1,4-dioxane obtained with the sample solution
A'_b: Peak area of 1,4-dioxane obtained with the standard solution

Head-space injection conditions—
Equilibration temperature in vial: A constant temperature of about 80°C.
Equilibration time in vial: 30 minutes.
Carrier gas: Helium.
Injection volume of sample: 1.0 mL.
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused silica column 0.53 mm in inside diameter and 50 m in length, coated the inside surface with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography 5 μm in thickness.
Column temperature: Inject at a constant temperature of about 70°C, rise the temperature at the rate of 10°C per minute to 250°C, and maintain the temperature at 250°C for 5 minutes.
Injection port temperature: A constant temperature of about 85°C.
Detector temperature: A constant temperature of about 250°C.
Carrier gas: Helium.
Flow rate: 4.0 mL per minute.
Split ratio: 1:3.5.
System suitability—
System performance: Introduce 0.100 g of acetaldehyde in a 100-mL volumetric flask, and add water to make 100 mL. To exact 1 mL of this solution add water to make exactly 100 mL. Transfer exactly 2 mL of this solution and exactly 2 mL of ethylene oxide stock solution into a 10-mL headspace vial, seal the vial immediately with a fluororesin coated silicon septum and an aluminum cap. Mix carefully, and use the content as the solution for system suitability test. When perform the test with ◆the standard solution and◆ the solution for system suitability test under the above conditions, acetaldehyde, ethylene oxide and 1,4-dioxane are eluted in this order, and the resolution between the peaks of acetaldehyde and ethylene oxide is not less than 2.0.
(3)   Peroxide value—Introduce about 10 g of Polysorbate 80, accurately weighed, into a 100-mL beaker, dissolve in 20 mL of acetic acid (100). Add 1 mL of saturated potassium iodide solution and allow to stand for 1 minute. Add 50 mL of fleshly boiled and cooled water, and titrate ⟨2.50⟩ with 0.01 mol/L sodium thiosulfate VS, while stirring with a

magnetic stirrer (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction. Calculate peroxide value by the following equation: not more than 10.0.

$$\text{Peroxide value} = (a - b) \times 10/M$$

M: Amount (g) of Polysorbate 80 taken
a: Volume (mL) of 0.01 mol/L sodium thiosulfate VS required for sample determination
b: Volume (mL) of 0.01 mol/L sodium thiosulfate VS required for blank determination

Water *<2.48>* Not more than 3.0% (1 g, volumetric titration, direct titration).

Residue on ignition Heat a quartz or platinum crucible to redness for 30 minutes, allow to cool in a desiccator (silica gel or other appropriate desiccants), and weigh accurately. Evenly distribute 2.00 g of Polysorbate 80 in the crucible, dry at 100 – 105°C for 1 hour, ◆and gradually heat with as lower temperature as possible to carbonize completely.◆ Then after igniting to constant mass in an electric furnace at 600 ± 25°C, allow the crucible to cool in a desiccator, and weigh the mass accurately. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up the ash with hot water, filter through a filter paper for quantitative analysis, and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness, and ignite to constant mass: not more than 0.25%.

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

Potash Soap

カリ石ケン

Potash Soap contains not less than 40.0% as fatty acids.

Method of preparation

Fixed oil	470 mL
Potassium Hydroxide	a sufficient quantity
Water, Purified Water or Purified	
Water in Containers	a sufficient quantity
	To make 1000 g

Dissolve Potassium Hydroxide, in required quantity for saponification, in Water, Purified Water or Purified Water in Containers, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol if necessary, stir thoroughly, heat in a water bath, and continue the saponification. After complete saponification, add Water, Purified Water or Purified Water in Containers to make 1000 g.

Description Potash Soap occurs as a yellow-brown, transparent, unctuous, soft mass, having a characteristic odor.
It is freely soluble in water and in ethanol (95).

Purity Silicic acid and alkalinity—Dissolve 10 g of Potash Soap in 30 mL of ethanol (95), and add 0.50 mL of 1 mol/L hydrochloric acid VS: no turbidity is produced. Add 1 drop of phenolphthalein TS to this solution: no red color develops.

Assay Weigh accurately about 5 g of Potash Soap, dissolve

in 100 mL of hot water, and transfer to a separator. Acidify the mixture with dilute sulfuric acid, and cool. Extract the solution with 50-mL, 40-mL, and 30-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10-mL portions of water until the washing contains no acid. Transfer the diethyl ether solution to a tared flask, evaporate diethyl ether on a water bath at a temperature as low as possible. Dry the residue at 80°C to constant mass, and weigh as fatty acids.

Containers and storage Containers—Tight containers.

Potassium Bromide

臭化カリウム

KBr: 119.00

Potassium Bromide, when dried, contains not less than 99.0% of potassium bromide (KBr).

Description Potassium Bromide occurs as colorless or white crystals, granules or crystalline powder. It is odorless.
It is freely soluble in water and in glycerin, soluble in hot ethanol (95), and slightly soluble in ethanol (95).

Identification A solution of Potassium Bromide (1 in 10) responds to Qualitative Tests *<1.09>* for potassium salt and for bromide.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Bromide in 3 mL of water: the solution is clear and colorless.
(2) Alkalinity—Dissolve 1.0 g of Potassium Bromide in 10 mL of water, add 0.10 mL of 0.05 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boiling, and cool: no color develops.
(3) Chloride—Make a calculation from the result obtained in the Assay: not more than 84.5 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Potassium Bromide.
(4) Sulfate *<1.14>*—Proceed with 2.0 g of Potassium Bromide, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).
(5) Iodide—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple to purple color develops in the chloroform layer.
(6) Bromate—Dissolve 1.0 g of Potassium Bromide in 10 mL of freshly boiled and cooled water, and add 0.1 mL of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.
(7) Heavy metals *<1.07>*—Proceed with 2.0 g of Potassium Bromide 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).
(8) Barium—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.
(9) Arsenic *<1.11>*—Prepare the test solution with 1.0 g of Potassium Bromide according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying *<2.41>* Not more than 1.0% (1 g, 110°C, 4 hours).

Assay Weigh accurately about 0.4 g of Potassium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and exactly measured 50 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.

$$\text{Each mL of 0.1 mol/L silver nitrate VS}$$
$$= 11.90 \text{ mg of KBr}$$

Containers and storage Containers—Tight containers.

Potassium Canrenoate

カンレノ酸カリウム
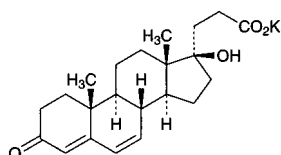
$C_{22}H_{29}KO_4$: 396.56
Monopotassium 17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylate
[2181-04-6]

Potassium Canrenoate, when dried, contains not less than 98.0% and not more than 102.0% of potassium canrenoate ($C_{22}H_{29}KO_4$).

Description Potassium Canrenoate occurs as a pale yellowish white to pale yellow-brown, crystalline powder.

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

Identification (1) Dissolve 2 mg of Potassium Canrenoate in 2 drops of sulfuric acid: an orange color develops. Observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Add 1 drop of acetic anhydride to this solution: the color of the solution changes to red.

(2) Determine the absorption spectrum of a solution of Potassium Canrenoate 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 Potassium Canrenoate, 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) The solution of Potassium Canrenoate (1 in 10) responds to Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: -71 – $-76°$ (after drying, 0.2 g, methanol, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Potassium Canrenoate in 20 mL of water: the pH of this solution is between 8.4 and 9.4.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Potassium Canrenoate in 5 mL of water: the solution is clear, and shows a pale yellow to light yellow color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Canrenoate 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 Potassium Canrenoate according to Method 3, and perform the test (not more than 2 ppm).

(4) Canrenone—Place 0.40 g of Potassium Canrenoate in a glass-stoppered centrifuge tube, cool in ice-water to a temperature not higher than 5°C, add 6 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 10.0) being cooled to a temperature not higher than 5°C to dissolve, and add 8 mL of water being cooled to a temperature not higher than 5°C. Add exactly 10 mL of chloroform, allow to stand for 3 minutes at a temperature not higher than 5°C, shake vigorously for 2 minutes, and centrifuge. Drain off the water layer, collect 5 mL of the chloroform layer, transfer to a glass-stoppered centrifuge tube containing 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 10.0) cooled to a temperature not higher than 5°C, and 4 mL of water cooled to a temperature not higher than 5°C, shake for 1 minute, and centrifuge. Drain off the water layer, pipet 2 mL of the chloroform layer, and add chloroform to make exactly 10 mL. Determine the absorbance of this solution at 283 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.67.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.2 g of Potassium Canrenoate, previously dried, dissolve in 75 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Use a solution of saturated potassium chloride-acetic acid (100) as the internal liquid.). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 39.66 \text{ mg of } C_{22}H_{29}KO_4$$

Containers and storage Containers—Tight containers.

Potassium Carbonate

炭酸カリウム

K_2CO_3: 138.21

Potassium Carbonate, when dried, contains not less than 99.0% of potassium carbonate (K_2CO_3).

Description Potassium Carbonate occurs as white granules or powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (95).

A solution of Potassium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

Identification A solution of Potassium Carbonate (1 in 10) responds to Qualitative Tests <1.09> for potassium salt and for carbonate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Potassium Carbonate in 2 mL of water and 6 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the

residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 6 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to 50 mL (not more than 20 ppm).

(3) Sodium—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water, and perform the test as directed under Flame Coloration Test <1.04> (1): no persisting yellow color is produced.

(4) Arsenic <1.11>—Prepare the test solution with 0.5 g of Potassium Carbonate, according to Method 1, and perform the test (not more than 4 ppm).

Loss on drying <2.41>   Not more than 1.0% (3 g, 180°C, 4 hours).

Assay   Dissolve about 1.5 g of Potassium Carbonate, previously dried and accurately weighed, in 25 mL of water, titrate with 0.5 mol/L sulfuric acid VS until the blue color of the solution changes to yellow-green, boil cautiously, then cool, and titrate <2.50> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

<div align="center">

Each mL of 0.5 mol/L sulfuric acid VS
= 69.11 mg of K_2CO_3

</div>

Containers and storage   Containers—Tight containers.

Potassium Chloride

塩化カリウム

KCl: 74.55

Potassium Chloride, when dried, contains not less than 99.0% of potassium chloride (KCl).

Description   Potassium Chloride occurs as colorless or white crystals or crystalline 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.

A solution of Potassium Chloride (1 in 10) is neutral.

Identification   A solution of Potassium Chloride (1 in 50) responds to Qualitative Tests <1.09> for potassium salt and for chloride.

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Potassium Chloride in 5 mL of water: the solution is clear and colorless.

(2) Acidity and alkalinity—Dissolve 5.0 g of Potassium Chloride in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: no red color develops. Then add 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Bromide—Dissolve 1.0 g of Potassium Chloride in water to make 100 mL. To 5 mL of the solution add 3 drops of dilute hydrochloric acid and 1 mL of chloroform, and add 3 drops of sodium toluensulfonchloramide TS dropwise while shaking: no yellow to yellow-red color develops in the chloroform layer.

(4) Iodide—Dissolve 0.5 g of Potassium Chloride in 10 mL of water, add 3 drops of iron (III) chloride TS and 1 mL of chloroform, shake, allow to stand for 30 minutes, and shake again: no red-purple to purple color develops in the chloroform layer.

(5) Heavy metals <1.07>—Proceed with 4.0 g of Potassium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(6) Calcium and magnesium—Dissolve 0.20 g of Potassium Chloride in 20 mL of water, add 2 mL of ammonia TS, 2 mL of ammonium oxalate TS and 2 mL of disodium hydrogenphosphate TS, and then allow to stand for 5 minutes: no turbidity is produced.

(7) Sodium—Dissolve 1.0 g of Potassium Chloride in 20 mL of water, and perform the Flame Coloration Test <1.04> (1): no persistent, yellow color develops.

(8) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Chloride according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <2.41>   Not more than 0.5% (1 g, 130°C, 2 hours).
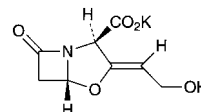
Assay   Weigh accurately about 0.2 g of Potassium Chloride, previously dried, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of fluorescein sodium TS).

<div align="center">

Each mL of 0.1 mol/L silver nitrate VS = 7.455 mg of KCl

</div>

Containers and storage   Containers—Tight containers.

Potassium Clavulanate

クラブラン酸カリウム

$C_8H_8KNO_5$: 237.25
Monopotassium (2*R*,5*R*)-3-[(1*Z*)-2-hydroxyethylidene]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate [*61177-45-5*]

Potassium Clavulanate is the potassium salt of a substance having β-lactamase inhibiting activity produced by the growth of *Streptomyces clavuligerus*.

It contains not less than 810 µg (potency) and not more than 860 µg (potency) per mg, calculated on the anhydrous basis. The potency of Potassium Clavulanate is expressed as mass (potency) of clavularic acid ($C_8H_9NO_5$: 199.16).

Description   Potassium Clavulanate occurs as a white to light yellowish white, crystalline powder.

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

It is hygroscopic.

Identification   (1)   To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30°C for 12 minutes. After cooling, 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 Potassium Clavulanate 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) Potassium Clavulanate responds to Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+53 - +63°$ (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Clavulanate according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

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

(3) Related substances—Dissolve 0.10 g of Potassium Clavulanate in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A 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 each peak other than clavulanic acid from the sample solution is not larger than the peak area of clavulanic acid from the standard solution, and the total area of the peaks other than clavulanic acid from the sample solution is not larger than 2 times of the peak area of clavulanic acid from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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 40°C.

Mobile phase A: Adjust the pH of 0.05 mol/L sodium dihydrogen phosphate TS to 4.0 with phosphoric acid.

Mobile phase B: A mixture of the mobile phase A 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 – 4	100	0
4 – 15	100 → 0	0 → 100
15 – 25	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 6 times as long as the retention time of clavulanic acid.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of clavulanic acid 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 10 mg each of Potassium Clavulanate and amoxycillin hydrate in 100 mL of the mobile phase A. When the procedure is run with 20 μL of this solution under the above operating conditions, clavulanic acid and amoxycillin are eluted in this order with the resolution between these peaks being not less than 8 and the num-

ber of theoretical plates of the peak of clavulanic acid is not less than 2500.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clavulanic acid is not more than 2.0%.

Water <2.48> Not more than 1.5% (5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Potassium Clavulanate and Lithium Clavulanate RS, equivalent to about 12.5 mg (potency), dissolve each in 30 mL of water, add exactly 5 mL of the internal standard solution and water 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 clavularic acid to that of the internal standard.

$$\text{Amount [μg (potency)] of clavularic acid (C}_8\text{H}_9\text{NO}_5)$$
$$= M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Lithium Clavulanate RS taken

*Internal standard solution—*Dissolve 0.3 g of sulfanilamide in 30 mL of methanol, and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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 1.36 g of sodium acetate trihydrate in 900 mL of water, adjust to pH 4.5 with diluted acetic acid (31) (2 in 5), and add 30 mL of methanol and water to make 1000 mL.

Flow rate: Adjust so that the retention time of clavularic acid 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, clavularic acid 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clavularic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Potassium Guaiacolsulfonate

グアヤコールスルホン酸カリウム
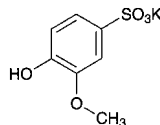
C$_7$H$_7$KO$_5$S: 242.29
Monopotassium 4-hydroxy-3-methoxybenzenesulfonate
[*16241-25-1*]

Potassium Guaiacolsulfonate contains not less than 98.5% of potassium guaiacolsulfonate (C$_7$H$_7$KO$_5$S), calculated on the anhydrous basis.

Description   Potassium Guaiacolsulfonate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a slightly bitter taste.

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

Identification   (1)   To 10 mL of a solution of Potassium Guaiacolsulfonate (1 in 100) add 2 drops of iron (III) chloride TS: a blue-purple color develops.

(2)   Dissolve 0.25 g of Potassium Guaiacolsulfonate in water to make 500 mL, and to 10 mL of this solution add phosphate buffer solution (pH 7.0) 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.

(3)   A solution of Potassium Guaiacolsulfonate (1 in 10) responds to Qualitative Tests <1.09> for potassium salt.

pH <2.54>   Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the solution is clear and colorless.

(2)   Sulfate <1.14>—Perform the test with 0.8 g of Potassium Guaiacolsulfonate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(3)   Heavy metals <1.07>—Proceed with 1.0 g of Potassium Guaiacolsulfonate 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 Potassium Guaiacolsulfonate according to Method 1, and perform the test (not more than 2 ppm).

(5)   Related substances—Dissolve 0.20 g of Potassium Guaiacolsulfonate in 200 mL of 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 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 obtained from these solutions by the automatic integration method: the total area of peaks other than potassium guaiacolsulfonate from the sample solution is not larger than the peak area of potassium guaiacolsulfonate from the

standard solution.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 279 nm).
Column: A stainless steel column 4 mm in inside diameter and 20 to 25 cm in length, packed with dimethylaminopropylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate TS and methanol (20:1).
Flow rate: Adjust so that the retention time of potassium guaiacolsulfonate is about 10 minutes.
Selection of column: Weigh 50 mg each of potassium guaiacolsulfonate and guaiacol, and dissolve in 50 mL of the mobile phase. Proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of guaiacol and potassium guaiacolsulfonate in this order with the resolution of these peaks being not less than 4.
Detection sensitivity: Adjust the sensitivity so that the peak height of potassium guaiacolsulfonate from 5 μL of the standard solution is not less than 10 mm.
Time span of measurement: About twice as long as the retention time of potassium guaiacolsulfonate.

Water <2.48>   3.0 – 4.5% (0.3 g, volumetric titration, direct titration).

Assay   Weigh accurately about 0.3 g of Potassium Guaiacolsulfonate, dissolve in 2.0 mL of formic acid, 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
= 24.23 mg of C$_7$H$_7$KO$_5$S

Containers and storage   Containers—Well-closed containers.
Storage—Light-resistant.

Potassium Hydroxide

水酸化カリウム

KOH: 56.11

Potassium Hydroxide contains not less than 85.0% of potassium hydroxide (KOH).

Description   Potassium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks and in other forms. It is hard and brittle, and shows a crystalline fracture.

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

It rapidly absorbs carbon dioxide in air.

It deliquesces in the presence of moisture.

Identification   (1)   A solution of Potassium Hydroxide (1 in 500) is alkaline.

(2)   A solution of Potassium Hydroxide (1 in 25) responds to Qualitative Tests <1.09> for potassium salt.

Purity   (1)   Clarity and color of solution—Dissolve 1.0 g of Potassium Hydroxide in 20 mL of water: the solution is clear and colorless.

(2)   Chloride <1.03>—Dissolve 2.0 g of Potassium Hy-

droxide in water to make 100 mL. To 25 mL of the solution add 8 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 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Potassium Hydroxide in 5 mL of water, add 7 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 30 ppm).

(4) Sodium—Dissolve 0.10 g of Potassium Hydroxide in 10 mL of dilute hydrochloric acid, and perform the test as directed under Flame Coloration Test ⟨*1.04*⟩ (1): no persistent yellow color develops.

(5) Potassium carbonate—The amount of potassium carbonate (K_2CO_3: 138.21) is not more than 2.0% when calculated by the following equation using B (mL) obtained in the Assay.

$$\text{Amount of potassium carbonate (mg)} = 138.21 \times B$$

Assay   Weigh accurately about 1.5 g of Potassium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate ⟨*2.50*⟩ with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount A (mL) of 0.5 mol/L sulfuric acid VS consumed, then add 2 drops of methyl orange TS, and titrate ⟨*2.50*⟩ again with 0.5 mol/L sulfuric acid VS until the solution changes to a persistent light red color. Record the amount B (mL) of 0.5 mol/L sulfuric acid VS consumed.

Calculate the amount potassium hydroxide (KOH) from the amount, A (mL) − B (mL).

$$\text{Each mL of 0.5 mol/L sulfuric acid VS} = 56.11 \text{ mg of KOH}$$

Containers and storage   Containers—Tight containers.

Potassium Iodide

ヨウ化カリウム

KI: 166.00

Potassium Iodide, when dried, contains not less than 99.0% of potassium iodide (KI).

Description   Potassium Iodide occurs as colorless or white crystals, or a white crystalline powder.

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

It is slightly deliquescent in moist air.

Identification   A solution of Potassium Iodide (1 in 20) responds to Qualitative Tests ⟨*1.09*⟩ for potassium salt and for iodide.

Purity   (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Iodide in 2 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 0.50 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color develops.

(3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Potassium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for 2 to 3 minutes, and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color develops. The solution has no more turbidity than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, and 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) Nitrate, nitrite and ammonium—Place 1.0 g of Potassium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert the absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on it. Heat the test tube carefully on a water bath for 15 minutes: the gas evolved does not turn red litmus paper to blue.

(5) Cyanide—Dissolve 0.5 g of Potassium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, then add 4 mL of hydrochloric acid: no green color develops.

(6) Iodate—Dissolve 0.5 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.

(7) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Potassium Iodide 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).

(8) Barium—Dissolve 0.5 g of Potassium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

(9) Sodium—Dissolve 1.0 g of Potassium Iodide in 10 mL of water, and perform the Flame Coloration Test ⟨*1.04*⟩ (1): a yellow color develops, but does not persist.

(10) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Potassium Iodide according to Method 1, and perform the test (not more than 5 ppm).

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

Assay   Weigh accurately about 0.5 g of Potassium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate ⟨*2.50*⟩ with 0.05 mol/L potassium iodate VS with shaking until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

$$\text{Each mL of 0.05 mol/L potassium iodate VS} = 16.60 \text{ mg of KI}$$

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

Potassium Permanganate

過マンガン酸カリウム

$KMnO_4$: 158.03

Potassium Permanganate, when dried, contains not less than 99.0% of potassium permanganate ($KMnO_4$).

Description   Potassium Permanganate occurs as dark purple crystals and has a metallic luster.

It is soluble in water.

A solution of Potassium Permanganate (1 in 1000) has a slightly sweet, astringent taste.

Identification   A solution of Potassium Permanganate (1 in 100) responds to Qualitative Tests <*1.09*> for permanganate.

Purity   (1)   Water-insoluble substances—Dissolve 2.0 g of Potassium Permanganate, previously powdered, in 200 mL of water. Filter the insoluble substances through a tared glass filter (G4), wash with water until the last washing shows no color, and dry at 105°C for 2 hours: the mass of the residue is not more than 4 mg.

(2)   Arsenic <*1.11*>—Dissolve 0.40 g of Potassium Permanganate in 10 mL of water, add 1 mL of sulfuric acid, add hydrogen peroxide (30) dropwise until the solution remains colorless, and evaporate on a sand bath nearly to dryness. Dissolve the residue in 5 mL of water, and perform the test with this solution as the test solution: the color produced is not more intense than the following color standard.

Color standard: To 10 mL of water add 1 mL of sulfuric acid and the same volume of hydrogen peroxide (30) as used for the preparation of the test solution. Evaporate the solution on a sand bath nearly to dryness, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and carry out the test with this solution in the same manner as the test solution (not more than 5 ppm).

Loss on drying <*2.41*>   Not more than 0.5% (1 g, silica gel, 18 hours).

Assay   Weigh accurately about 0.6 g of Potassium Permanganate, previously dried, dissolve in water to make exactly 200 mL, and use this solution as the sample solution. Pipet 25 mL of 0.05 mol/L oxalic acid VS into a 500-mL conical flask, add 200 mL of diluted sulfuric acid (1 in 20), and keep at a temperature between 30°C and 35°C. Transfer the sample solution to a buret. Add quickly 23 mL of the sample solution from the buret to the flask while shaking gently, and then allow the flask to stand until the red color disappears. Warm the mixture to a temperature between 55°C and 60°C, and continue the titration <*2.50*> slowly until the red color persists for 30 seconds.

Each mL of 0.05 mol/L oxalic acid VS
= 3.161 mg of $KMnO_4$

Containers and storage   Containers—Tight containers.

Potassium Sulfate

硫酸カリウム

K_2SO_4: 174.26

Potassium Sulfate, when dried, contains not less than 99.0% of potassium sulfate (K_2SO_4).

Description   Potassium Sulfate occurs as colorless crystals or a white, crystalline powder. It has a slightly saline, somewhat bitter taste.

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

Identification   A solution of Potassium Sulfate (1 in 20) responds to Qualitative Tests <*1.09*> for potassium salt and for sulfate.

Purity   (1)   Clarity and color of solution, and acid or alkali—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water: the solution is clear, colorless and neutral.

(2)   Chloride <*1.03*>—Perform the test with 0.5 g of Potassium Sulfate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3)   Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium 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).

(4)   Sodium—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water, and perform the test as directed under Flame Coloration Test <*1.04*> (1): no persistent yellow color develops.

(5)   Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Potassium Sulfate according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*>   Not more than 1.0% (1 g, 110°C, 4 hours).

Assay   Weigh accurately about 0.5 g of Potassium Sulfate, previously dried, boil with 200 mL of water and 1.0 mL of hydrochloric acid, and add gradually 8 mL of boiling barium chloride TS. Heat the mixture on a water bath for 1 hour, collect the precipitate, and wash the precipitate with water until the last washing shows no opalescence on the addition of silver nitrate TS. Dry, heat strongly to constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of potassium sulfate (K_2SO_4)
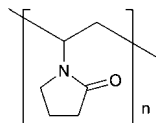= amount (mg) of barium sulfate ($BaSO_4$) × 0.747

Containers and storage   Containers—Well-closed containers.

Povidone

Polyvidone

Polyvinylpyrrolidone

ポビドン

$(C_6H_9NO)_n$
Poly[1-(2-oxopyrrolidin-1-yl)ethylene]
[*9003-39-8*]

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 (♦ ♦).

Povidone is a chain polymer of 1-vinyl-2-pyrrolidone.

It contains not less than 11.5% and not more than 12.8% of nitrogen (N: 14.01), calculated on the anhydrous basis.

It has a nominal K-value of not less than 10 and not more than 120.

The nominal K-value is shown on the label.

♦**Description** Povidone occurs as a white to slightly yellowish fine powder. It is odorless or has a faint, characteristic odor.

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

It is hygroscopic.♦

Identification **(1)** To 0.5 g of Povidone add 10 mL of water, and shake: it dissolves.

(2) Determine the infrared absorption spectrum of Povidone, previously dried at 105°C for 6 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 Povidone RS (previously dried at 105°C for 6 hours): both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Povidone in 20 mL of water: the pH of this solution is between 3.0 and 5.0 for Povidone having the nominal K-value of 30 or less, and between 4.0 and 7.0 for Povidone having the nominal K-value exceeding 30.

Purity ♦**(1)** Clarity and color of solution—Dissolve 1.0 g of Povidone in 20 mL of water: the solution is clear and colorless to pale yellow, or pale red.♦

♦**(2)** Heavy metals <1.07>—Proceed with 2.0 g of Povidone 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) Aldehydes—Weigh accurately about 1 g of Povidone and dissolve in 0.05 mol/L pyrophosphate buffer solution (pH 9.0) to make exactly 100 mL. Stopper, heat at 60°C for 60 minutes, allow to cool to room temperature, and use this solution as the sample solution. Separately, dissolve 0.140 g of acetaldehyde ammonia trimer trihydrate in water to make exactly 200 mL. Pipet 1 mL of this solution, add 0.05 mol/L pyrophosphate buffer solution (pH 9.0) to make exactly 100

mL, and use this solution as the standard solution. Measure exactly 0.5 mL each of the sample solution, standard solution and water, transfer to separate 1-cm cells, add 2.5 mL of 0.05 mol/L pyrophosphate buffer solution (pH 9.0) and 0.2 mL of β-nicotinamide adenine dinucleotide TS to each of these cells, mix and stopper tightly. Allow to stand for 2 to 3 minutes at 22 ± 2°C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the control solution. Determine the absorbances, A_{T1}, A_{S1} and A_{B1} of the subsequent solutions of the sample solution, the standard solution and water at 340 nm. Add 0.05 mL of aldehyde dehydrogenase TS to each of the cells, mix and stopper tightly. Allow to stand for 5 minutes at 22 ± 2°C. Determine the absorbances, A_{T2}, A_{S2} and A_{B2} of these solutions in the same manner as above, and calculate the content of aldehydes by the follwing equation: the content of aldehydes is not more than 500 ppm.

Content (ppm) of aldehydes [as acetaldehyde (CH₃CHO)]
$$= C/M \times \{(A_{T2} - A_{T1}) - (A_{B2} - A_{B1})\}/\{(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})\} \times 100,000$$

M: Amount (g) of Povidone taken, calculated on the anhydrous basis

C: Concentration (mg/mL) of acetaldehyde in the standard solution, using 0.72 as conversion factor for acetaldehyde ammonia trimer trihydrate to acetaldehyde

(4) 1-Vinyl-2-pyrrolidone—Weigh accurately about 0.25 g of Povidone, dissolve in a mixture of water and acetonitrile (9:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in a mixture of water and acetonitrile (9:1) to make exactly 100 mL. Pipet 1 mL of this solution and add a mixture of water and acetonitrile (9:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of water and acetonitrile (9:1) 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 areas, A_T and A_S, of 1-vinyl-2-pyrrolidone in each solution, and calculate the content of 1-vinyl-2-pyrrolidone by the following equation: it is not more than 10 ppm.

Content (ppm) of 1-vinyl-2-pyrrolidone
$$= 1/M \times A_T/A_S \times 2.5$$

M: Amount (g) of Povidone taken, calculated on the anhydrous basis

Operating conditions—
Detector: An ultraviolet spectrophotometer (detection wavelength: 235 nm).
Column: Stainless steel columns 4.0 mm in inside diameter and 10 mm in length, and 4.6 mm in inside diameter and 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter), and use them as a guard column and a separation column, respectively.
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of water and acetonitrile (9:1).
Flow rate: 1.0 mL per minutes.
System suitability—
System performance: Dissolve 10 mg of 1-vinyl-2-pyrrolidone and 0.5 g of vinyl acetate in 100 mL of methanol. To 1 mL of this solution add a mixture of water and acetonitrile (9:1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 1-

vinyl-2-pyrrolidone and vinyl acetate 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-vinyl-2-pyrrolidone is not more than 2.0%.

(5) Peroxides—Weigh exactly an amount of Povidone, equivalent to 4.0 g calculated on the anhydrous basis, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. To 25 mL of the sample solution add 2 mL of titanium (III) chloride-sulfuric acid TS, and mix. Allow to stand for 30 minutes, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding 2 mL of 13% sulfuric acid to 25 mL of the sample solution as a blank: the absorbance of the solution at 405 nm is not more than 0.35 (not more than 400 ppm, expressed as hydrogen peroxide).

(6) Hydrazine—Weigh exactly an amount of Povidone equivalent to 2.5 g calculated on the anhydrous basis, transfer to a 50-mL centrifuge tube, add 25 mL of water, and stir to dissolve. Add 500 μL of a solution of salicylaldehyde in methanol (1 in 20), stir and warm at 60°C for 15 minutes in a water bath. Allow to cool, add 2.0 mL of toluene, stopper tightly, shake vigorously for 2 minutes, centrifuge, and use the upper layer of the mixture as the sample solution. Separately, dissolve 90 mg of salicylaldazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene 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 dimethylsilanized silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and water (2:1) to a distance of about three-fourths of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the fluorescence of the spot from the sample solution corresponding to the spot having a Rf value of about 0.3 from the standard solution is not more intense than that of the spot from the standard solution (not more than 1 ppm).

(7) Formic acid—Weigh accurately about 2 g of Pivodone, dissolve in water to make exactly 100 mL, and use this solution as the sample stock solution. Transfer a strongly acidic ion exchange resin (H type) for column chromatography previously suspended in water to a column of about 8 mm in inside diameter to give a packing depth of about 20 mm in length, and keep the resin layer constantly immersed in water. Pour 5 mL of water to the column, and adjust the flow rate about 1 mL per minute. When the level of the water comes down to near the top of the resin layer, put the sample stock solution into the column, discard the first 2 mL of the eluent, take 1.5 mL of the subsequent eluent, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of formic acid, dissolve in 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 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 formic acid in each solution. Calculate the content of formic acid by the following equation: it is not more than 0.5%.

$$\text{Content of formic acid(\%)} = M_S/M_T \times A_T/A_S$$

M_S: Amount (g) of formic acid taken

M_T: Amount (g) of Povidone taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 7.8 mm in inside diameter and 300 mm in length, packed with strongly acidic ion exchange resin for liquid chromatography (9 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Diluted perchloric acid (1 in 700).

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 number of theoretical plates and the symmetry factor of the peak of formic acid are not less han 1000 and 0.5 to 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 formic acid is not more than 2.0%.

(8) 2-Pyrrolidone—Weigh accurately about 0.5 g of Povidone, dissolve in a mixture of water and methanol for liquid chromatography (19:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 0.150 g of 2-pyrrolidone in a mixture of water and methanol for liquid chromatography (19:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of water and methanol for liquid chromatography (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> according to the following conditions, and determine the peak areas, A_T and A_S, of 2-pyrrolidone in each solution. Calculate the content of 2-pyrrolidone by the following equation: not more than 3.0%.

$$\text{Content (\%) of 2-pyrrolidone} = 1/M \times A_T/A_S \times 0.3$$

M: The amount (g) of Povidone taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: Stainless steel columns 4.0 mm in inside diameter and 10 mm in length, and 4.6 mm in inside diameter and 150 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter), and use them as a guard column and a separation column, respectively.

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

Mobile phase: A mixture of water and methanol for liquid chromatography (19 : 1).

Flow rate: 0.8 mL per min.

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 2-pyrrolidone 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 2-pyrrolidone is not more than 2.0%.

Water <2.48> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

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

K-value Weigh accurately an amount of Povidone, calculated on the anhydrous basis, specified in the table below according to the nominal K-value, dissolve in water to make exactly 100 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Perform the test with the sample solution and with water at 25°C as directed in Method 1 under Viscosity Determination <2.53>, and calculate the K-value by the following formula.

$$K = \frac{1.5 \log v_{rel.} - 1}{0.15 + 0.003\,c} + \frac{\sqrt{300\,c \log v_{rel.} + (c + 1.5\,c \log v_{rel.})^2}}{0.15\,c + 0.003\,c^2}$$

  c: Mass (g) of Povidone in 100 mL of the solution, calculated on the anhydrous basis

  $v_{rel.}$: Kinematic viscosity of the sample solution relative to that of water

Nominal K-value	Amount (g) calculated on anhydrous basis
Not more than 18	5.00
More than 18 and more than 95	1.00
More than 95	0.10

The K-value is not less than 85% and not more than 115.0% of the nominal K-value when the nominal K-value is not more than 15, and the K-value is not less than 90.0% and not more than 108.0% of the nominal K-value when the nominal K-value is more than 15.

Assay Weigh accurately about 0.1 g of Povidone, and place in a Kjeldahl flask. Add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and wash down any adhering sample from the neck of the flask with a small amount of water. Add 7 mL of sulfuric acid allowing to flow down the inside wall of the flask. Heat the flask gradually over a free flame until the solution has a clear, yellow-green color and the inside wall of the flask is free from a carbonaceous material, and then heat for further 45 minutes. After cooling, add cautiously 20 mL of water, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of sodium hydroxide (2 in 5) through the funnel, rinse cautiously the funnel with 10 ml of water, immediately close the clamp attached to the rubber tube, then start the distillation with steam to get 80 to 100 mL of the distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate <2.50> the distillate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

  Each mL of 0.025 mol/L sulfuric acid VS
    = 0.700 mg of N

◆**Containers and storage** Containers—Tight containers.◆

Povidone-Iodine

ポビドンヨード
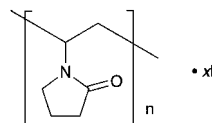
$(C_6H_9NO)_n.x I$
Poly[1-(2-oxopyrrolidin-1-yl)ethylene] iodine
[*25655-41-8*]

Povidone-Iodine is a complex of iodine with 1-vinyl-2-pyrrolidone polymer.

It contains not less than 9.0% and not more than 12.0% of available iodine (I: 126.90), and not less than 9.5% and not more than 11.5% of nitrogen (N: 14.01), calculated on the dried basis.

Description Povidone-Iodine occurs as a dark red-brown powder. It has a faint, characteristic odor.

It is freely soluble in water and in ethanol (99.5).

The pH of a solution obtained by dissolving 1.0 g of Povidone-Iodine in 100 mL of water is between 1.5 and 3.5.

Identification (1) To 10 mL of diluted starch TS (1 in 10) add 1 drop of a solution of Povidone-Iodine (1 in 10): a deep blue color develops.

(2) To 1 mL of a solution of Povidone-Iodine (1 in 100) add 1 mL of sodium thiosulfate TS, and add 1 mL of ammonium thiocyanate-cobalt (II) nitrate TS and 2 drops of 1 mol/L hydrochloric acid TS: a blue color develops, and a blue precipitate is gradually formed.

Purity (1) Clarity and color of solution—Dissolve 0.30 g of Povidone-Iodine in 100 mL of water: the solution is clear and brown.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Povidone-Iodine 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 Povidone-Iodine according to Method 4, and perform the test (not more than 2 ppm).

(4) Iodide ion—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 100 mL of water, and add sodium hydrogensulfite TS until the color of iodine completely disappears. To this solution add exactly 25 mL of 0.1 mol/L silver nitrate VS, shake well with 10 mL of nitric acid, titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS until the solution develops a red-brown color, and calculate the total amount of iodine (indicator: 1 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

  Each mL of 0.1 mol/L ammonium thiocyanate VS
    = 12.69 mg of I

Obtain the amount of iodide ion, calculated on the dried basis, by deducting the amount (%) of available iodine from the total amount (%) of iodine: not more than 6.6%.

Loss on drying <2.41> Not more than 8.0% (1 g, 100°C, 3 hours).

Residue on ignition <2.44> Not more than 0.05% (5 g).

Assay (1) Available iodine—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 30 mL of water, and

titrate <*2.50*> with 0.02 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).
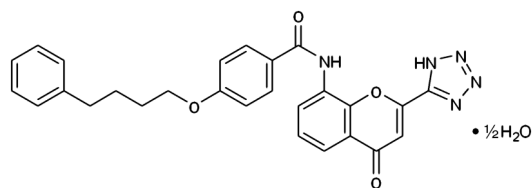
> Each mL of 0.02 mol/L sodium thiosulfate VS
> = 2.538 mg of I

(2) Nitrogen—Weigh accurately about 20 mg of Povidone-Iodine, and perform the test as directed under Nitrogen Determination <*1.08*>.

Containers and storage Containers—Tight containers.

Pranlukast Hydrate

プランルカスト水和物

$C_{27}H_{23}N_5O_4.^{1}/_{2}H_2O$: 490.51
N-[4-Oxo-2-(1*H*-tetrazol-5-yl)–4*H*-chromen-8-yl]-4-(4-phenylbutyloxy)benzamide hemihydrate
[*150821-03-7*]

Pranlukast Hydrate contains not less than 98.0% and not more than 101.0% of pranlukast ($C_{27}H_{23}N_5O_4$: 481.50), calculated on the anhydrous basis.

Description Pranlukast Hydrate occurs as a white to light yellow, crystalline powder.

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

Melting point: about 233°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Pranlukast Hydrate 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 Pranlukast 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 Pranlukast 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 Pranlukast RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <*1.07*>—Suspend 1.0 g of Pranlukast Hydrate in 10 mL of *N,N*-dimethylformamide, proceed according to Method 4, and perform the test. Prepare the control solution with 10 mL of *N,N*-dimethylformamide in the same manner as preparation of the test solution, and add 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Suspend 1.0 g of Pranlukast Hydrate in 10 mL of *N,N*-dimethylformamide, then proceed according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 20 mg of Pranlukast Hydrate in 50 mL of a mixture of acetonitrile and dimethylsulfoxide (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and dimethylsulfoxide (3:1) 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, having the relative retention time about 1.5 to pranlukast, obtained from the sample solution is not larger than 1/2 times that of pranlukast obtained from the standard solution, the area of the peak other than pranlukast and the peak mentioned above from the sample solution is not larger than 1/5 times that of pranlukast from the standard solution, and the total area of the peaks other than pranlukast from the sample solution is not larger than the peak area of pranlukast 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 pranlukast, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add a mixture of acetonitrile and dimethylsulfoxide (3:1) to make exactly 50 mL. Confirm that the peak area of pranlukast 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: 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 pranlukast 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 pranlukast is not more than 2.0%.

Water <*2.48*> 1.5 – 2.2% (50 mg, coulometric titration).

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

Assay Weigh accurately about 20 mg each of Pranlukast Hydrate and Pranlukast RS (separately determine the water <*2.48*> in the same manner as Pranlukast Hydrate), dissolve them separately in a mixture of acetonitrile and dimethylsulfoxide (3:1) to make exactly 50 mL. To exactly 5 mL each of these solutions add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 4 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 pranlukast to that of the internal standard.

> Amount (mg) of pranlukast ($C_{27}H_{23}N_5O_4$)
> = $M_S \times Q_T/Q_S$

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

Internal standard solution—A solution of isoamyl para-hydroxybenzoate in a mixture of acetonitrile and dimethylsulfoxide (3:1) (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 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 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, acetonitrile and methanol (5:5:1).

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

System suitability—

System performance: When the procedure is run with 4 μL of the standard solution under the above operating conditions, pranlukast 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 4 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pranlukast to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Pranoprofen

プラノプロフェン

and enantiomer
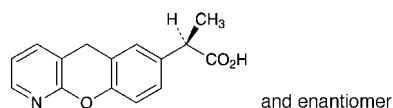
$C_{15}H_{13}NO_3$: 255.27
(2*RS*)-2-(10*H*-9-Oxa-1-azaanthracen-6-yl)propanoic acid
[*52549-17-4*]

Pranoprofen, when dried, contains not less than 98.5% of pranoprofen ($C_{15}H_{13}NO_3$).

Description   Pranoprofen occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in acetonitrile, in ethanol (95) and in acetic anhydride, very slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Pranoprofen in *N,N*-dimethylformamide (1 in 30) shows no optical rotation.

Identification   **(1)**   Dissolve 0.02 g of Pranoprofen in 1 mol/L hydrochloric acid TS to make 100 mL, and 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: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2)   Determine the infrared absorption spectrum of Pranoprofen 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>   186 – 190°C

Purity   **(1)**   Chloride <1.03>—Dissolve 0.5 g of Pranoprofen in 40 mL of methanol, and 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 as follows. To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of methanol, 6 mL of dilute nitric acid and water

to make 50 mL (not more than 0.021%).

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

(3)   Related Substances—Dissolve 50 mg of Pranoprofen 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. Determine each peak area from both solutions by the automatic integration method: the each area of the peaks other than pranoprofen from the sample solution is not larger than the peak area of pranoprofen from the standard solution, and the total peak area of them is not larger than 2 times the peak area of pranoprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 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: Dissolve 7.02 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with perchloric acid. To 2 volumes of this solution add 1 volume of acetonitrile.

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

Selection of column: Dissolve 4 mg each of Pranoprofen and ethyl parahydroxybenzoate in 200 mL of the mobile phase. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pranoprofen and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of pranoprofen from 10 μL of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About three times as long as the retention time of pranoprofen.

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).

Assay   Weigh accurately about 0.4 g of Pranoprofen, 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). Perform a blank determination, and make any necessary correction.
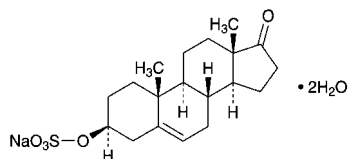
Each mL of 0.1 mol/L perchloric acid VS
= 25.53 mg of $C_{15}H_{13}NO_3$

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

Prasterone Sodium Sulfate Hydrate

プラステロン硫酸エステルナトリウム水和物

$C_{19}H_{27}NaO_5S.2H_2O$: 426.50
Monosodium 17-oxoandrost-5-en-3β-yl sulfate dihydrate
[*1099-87-2*, anhydride]

Sodium Prasterone Sulfate Hydrate contains not less than 98.0% of prasterone sodium sulfate ($C_{19}H_{27}NaO_5S$: 390.47), calculated on the dried basis.

Description Prasterone Sodium Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless.

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

The pH of a solution of 1.0 g of Prasterone Sodium Sulfate Hydrate in 200 mL of water is between 4.5 and 6.5.

Melting point: about 160°C (with decomposition, after drying).

Identification (1) Dissolve 0.01 g of Prasterone Sodium Sulfate Hydrate in 4 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of a solution of sodium hydroxide (1 in 8): a red-purple color develops, and gradually changes to brown.

(2) To 10 mL of a solution of Prasterone Sodium Sulfate Hydrate (1 in 200) add 0.5 mL of bromine TS: the color of bromine TS immediately disappears.

(3) Determine the infrared absorption spectrum of Prasterone Sodium Sulfate Hydrate 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.

(4) A solution of Prasterone Sodium Sulfate Hydrate (1 in 200) responds to the Qualitative Tests <1.09> for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +10.7 – +12.1° (0.73 g calculated on the dried basis, methanol, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.25 g of Prasterone Sodium Sulfate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Prasterone Sodium Sulfate Hydrate in 20 mL of acetone and 20 mL of water, 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 0.30 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 0.011%).

(3) Sulfate <1.14>—To 1.2 g of Prasterone Sodium Sulfate Hydrate add 20 mL of water, shake vigorously for 5 minutes, and filter. To 10 mL of the filtrate add 20 mL of acetone, 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 0.032%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Prasterone Sodium Sulfate 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).

(5) Related substances—Dissolve 0.10 g of Prasterone Sodium Sulfate 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and water (75:22:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of sulfuric acid and ethanol (95) (1:1) on the plate, 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> 8.0 – 9.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Weigh accurately about 0.25 g of Prasterone Sodium Sulfate Hydrate, dissolve in 30 mL of water. Apply this solution to a chromatographic column 10 mm in inside diameter, previously prepared by pouring 5 mL of strongly acidic ion-exchange resin (H type) for column chromatography, and elute at the rate of 4 mL per minute. Wash the chromatographic column with 100 mL of water, combine the washings with above effluent solution, and titrate <2.50> with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS
= 19.52 mg of $C_{19}H_{27}NaO_5S$

Containers and storage Containers—Tight containers.

Pravastatin Sodium

プラバスタチンナトリウム
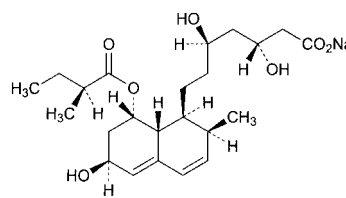
$C_{23}H_{35}NaO_7$: 446.51
Monosodium (3R,5R)-3,5-dihydroxy-7-{(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-[(2S)-2-methylbutanoyloxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl}heptanoate
[*81131-70-6*]

Pravastatin Sodium contains not less than 98.5% and not more than 101.0% of pravastatin sodium ($C_{23}H_{35}NaO_7$), calculated on the anhydrous and residual solvent-free basis.

Description Pravastatin Sodium occurs as a white to yellowish white, powder or crystalline powder.

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

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Pravastatin Sodium (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 Pravastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2970 cm^{-1}, 2880 cm^{-1}, 1727 cm^{-1} and 1578 cm^{-1}.

(3) Dissolve 50 mg of Pravastatin Sodium in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 24 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium 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 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 ethyl acetate, ethanol (99.5) and acetic acid (100) (80:16:1) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the color tone and the Rf value of the principal spot with the sample solution are not different with them of the spot with the standard solution.

(4) A solution of Pravastatin Sodium (1 in 10) responds to Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +153 – +159° (0.1 g calculated on the anhydrous and residual solvent-free basis, water, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Pravastatin Sodium in 20 mL of freshly boiled and cooled water is between 7.2 and 8.2.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pravastatin Sodium 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 Pravastatin Sodium in 100 mL of a mixture of water and methanol (11:9), and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of water and methanol (11:9) 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 other than pravastatin from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin from the sample solution is not larger than the peak area of pravastatin from the standard solution. Keep the sample solution and standard solution at not over than15°C.
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 pravastatin, 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 methanol (11:9) to make exactly 50 mL. Confirm that the peak area of pravastatin 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: Dissolve 5 mg of pravastatin sodium in 50 mL of the mixture of water and methanol (11:9). 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 pravastatin are not less than 3500 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 pravastatin is not more than 2.0%.

Water <2.48> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Pravastatin Sodium, and dissolve in a mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (11:9) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (previously determine the water <2.48> with 0.5 g by direct titration in volumetric titration) dissolve in the mixture of water and methanol (11:9) to make exactly 25 mL. Proceed with exactly 10 mL of this solution in the same manner for the preparation of the sample solution, and use the solution so obtained 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 pravastatin to that of the internal standard.

$$\text{Amount (mg) of pravastatin sodium } (C_{23}H_{35}NaO_7)$$
$$= M_S \times Q_T/Q_S \times 4 \times 1.052$$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

*Internal standard solution—*A solution of ethyl parahydroxybenzoate in the mixture of water and methanol (11:9) (3 in 4000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 238 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, methanol, acetic acid (100) and triethylamine (550:450:1:1).
Flow rate: Adjust so that the retention time of pravastatin is about 21 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 pravastatin 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 pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Pravastatin Sodium Fine Granules

プラバスタチンナトリウム細粒

Pravastatin Sodium Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$: 446.51).

Method of preparation   Prepare as directed under Granules, with Pravastatin Sodium.

Identification   To an amount of Pravastatin Sodium Fine Granules, equivalent to 10 mg of Pravastatin Sodium, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 237 nm and 241 nm.

Purity   Related substances—The sample solution and the standard solution are stored at not exceeding 5°C after preparation. To an amount of Pravastatin Sodium Fine Granules, equivalent to 25 mg of Pravastatin Sodium, add 25 mL of a mixture of water and methanol (1:1), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) 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 the peaks, having the relative retention time of about 0.36 and about 1.9 to pravastatin, obtained from the sample solution is not larger than 1/2 times and 3 times the peak area of pravastatin obtained from the standard solution, respectively, the area of the peak other than pravastatin and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin from the sample solution is not larger than 4.5 times the peak area of pravastatin from the standard solution. For the area of the peaks, having the relative retention time of about 0.28, about 0.36 and about 0.88 to pravastatin, multiply their relative response factors, 1.16, 1.72 and 1.22, respectively.
Operating conditions—
Detector: An ultraviolet spectrophotometer (wavelength: 238 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: A mixture of water, methanol, acetic acid (100) and triethylamine (750:250:1:1).
Mobile phase B: A mixture of methanol, water, acetic acid

(100) and triethylamine (650:350:1:1).
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 – 50	50	50
50 – 75	50 → 0	50 → 100

Flow rate: 1.3 mL per minute.
Time span of measurement: For 75 minutes after injection, 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 methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin 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 pravastatin are not less than 3500 and not more than 1.6, 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 pravastatin is not more than 1.5%.

Uniformity of dosage units <6.02>   Perform the test according to the following method: the Pravastatin Sodium 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 Pravastatin Sodium Fine Granules add exactly V mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate add a mixture of water and methanol (1 in 1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)
= $M_S \times Q_T/Q_S \times V/100 \times 1.052$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 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 15 minutes of Pravastatin Sodium Fine Granules is not less than 80%.
Start the test with an accurately weighed amount of Pravastatin Sodium Fine Granules, equivalent to about 5 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), 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 23 mg of Pravastatin

1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <2.48> in the same manner as Pravastatin Sodium), and dissolve in water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{S1}, at 238 nm and A_{T2} and A_{S2} at 265 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 pravastatin sodium ($C_{23}H_{35}NaO_7$)

$$= M_S/M_T \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2})$$
$$\times 1/C \times 27 \times 0.806$$

M_S: Amount (mg) of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS taken, calculated on the anhydrous basis

M_T: Amount (g) of Pravastatin Sodium Fine Granules taken

C: Labeled amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$) in 1 g

Assay Weigh accurately an amount of Pravastatin Sodium Fine Granules, equivalent to about 5 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), add exactly 20 mL of the internal standard solution, agitate for 15 minute with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 32 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water <2.48> in the same manner as Pravastatin Sodium), and dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) 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 pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$$= M_S \times Q_T/Q_S \times 1/5 \times 1.052$$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Pravastatin Sodium.
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 pravastatin 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 ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Pravastatin Sodium Solution

プラバスタチンナトリウム液

Pravastatin Sodium Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$: 446.51).

Method of preparation Prepare as directed under Liquids and Solutions for Oral Administration, with Pravastatin Sodium.

Identification Pass a volume of Pravastatin Sodium Solution, equivalent to 1 mg of Pravastatin Sodium, through a column [5.5 mm in inside diameter, packed with 30 mg of divinylbenzene-*N*-vinyl pyrrolidone copolymer for column chromatography (30 μm in particle size), and washed with 1 mL of methanol and 1 mL of water]. Then wash with 1 mL of water, and elute with 1 mL of methanol. To 0.1 mL of the eluate add water to make 10 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 237 nm and 241 nm.

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

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To a volume of Pravastatin Sodium Solution, equivalent to 2 mg of Pravastatin Sodium, add a mixture of methanol and water (5:3) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) 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 peaks, having the relative retention time about 0.24 and about 0.85 to pravastatin, obtained from the sample solution is not larger than 2 times the peak area of pravastatin obtained from the standard solution, the area of the peak other than pravastatin and the peaks mentioned above from the sample solution is not larger than 3/10 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin from the sample solution is not larger than 3.5 times the peak area of pravastatin 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 pravasatin, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin 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 pravastatin are not less than 3400 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 pravastatin is not more than 2.5%.

Uniformity of dosage units *<6.02>* The solution in single-dose packages meet the requirement of the Mass variation test.

Microbial limit *<4.05>* The acceptance criteria of TAMC and TYMC are 10^2 CFU/mL and 10^1 CFU/mL, respectively. *Escherichia coli* is not observed.

Assay To an exact volume of Pravastatin Sodium Solution, equivalent to 2 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), add exactly 5 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water *<2.48>* in the same manner as Pravastatin Sodium), and dissolve in a solution of disodium hydrogen phosphate dodecahydrate (1 in 200) to make exactly 50 mL. Pipet 6 mL of this solution, add exactly 5 mL of the internal standard solution, 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. Calculate the ratios, Q_T and Q_S, of the peak area of pravastatin to that of the internal standard.

$$\text{Amount (mg) of pravastatin sodium} = M_S \times Q_T/Q_S \times 3/25 \times 1.052$$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (3 in 10,000).
Operating conditions—
Detector: An ultraviolet spectrophotometer (wavelength: 238 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: A mixture of water, methanol, acetic acid (100) and triethylamine (500:500:1:1).
Flow rate: Adjust so that the retention time of pravastatin is about 20 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 pravastatin 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 ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pravastatin Sodium Tablets

プラバスタチンナトリウム錠

Pravastatin Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pravastatin sodium ($C_{23}H_{35}NaO_7$: 446.51).

Method of preparation Prepare as directed under Tablets, with Pravastatin Sodium.

Identification To a quantity of powdered Pravastatin Sodium Tablets, equivalent to 10 mg of Pravastatin Sodium, add 20 mL of water, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, and add water to 1 mL of the subsequent filtrate to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 237 nm and 241 nm.

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To an amount of powdered Pravastatin Sodium Tablets, equivalent to 50 mg of Pravastatin Sodium, add 40 mL of a mixture of water and methanol (1:1), agitate with the aid of ultrasonic waves, then add a mixture of water and methanol (1:1) to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) 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 the peaks, having the relative retention time about 0.36 and about 1.9 to pravastatin obtained from the sample solution is not larger than 3/10 times and 2 times the peak area of pravastatin obtained from the standard solution, respectively, the area of the peak other than pravastatin and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin from the sample solution is not larger than 3 times the peak area of pravastatin from the standard solution. For the area of the peaks, having the relative retention time about 0.28, about 0.36 and about 0.88, multiply their relative response factors, 1.16, 1.72 and 1.22, respectively.
Operating conditions—
Detector: An ultraviolet spectrophotometer (wavelength: 238 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: A mixture of water, methanol, acetic acid (100) and triethylamine (750:250:1:1).
Mobile phase B: A mixture of methanol, water, acetic acid (100) and triethylamine (650:350:1:1).
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 – 50	50	50
50 – 75	50 → 0	50 → 100

Flow rate: 1.3 mL per minute.

Time span of measurement: For 75 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin 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 pravastatin are not less than 3500 and not more than 1.6, 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 pravastatin is not more than 1.5%.

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 Pravastatin Sodium Tablets add exactly V mL of the internal standard solution so that each mL contains 0.25 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. To 2 mL of the supernatant liquid add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$$= M_S \times Q_T/Q_S \times V/100 \times 1.052$$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 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 Pravastatin Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Pravastatin 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 5.5 μg of pravastatin ($C_{23}H_{36}O_7$), and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water *<2.48>* in the same manner as Pravastatin Sodium), and dissolve in water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{S1}, at

238 nm and A_{T2} and A_{S2} at 256 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 pravastatin sodium ($C_{23}H_{35}NaO_7$)
$$= M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2})$$
$$\times V'/V \times 1/C \times 27 \times 0.806$$

M_S: Amount (mg) of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS taken, calculated on the anhydrous basis

C: Labeled amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Pravastatin Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of pravastatin sodium ($C_{23}H_{35}NaO_7$), add exactly 40 mL of the internal standard solution, agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add a mixture of water and methanol (1:1) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 32 mg of Pravastatin 1,1,3,3-Tetramethylbutylammonium RS (separately determine the water *<2.48>* in the same manner as Pravastatin Sodium), and dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) 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. Calculate the ratios, Q_T and Q_S, of the peak area of pravastatin to that of the internal standard.

Amount (mg) of pravastatin sodium ($C_{23}H_{35}NaO_7$)
$$= M_S \times Q_T/Q_S \times 2/5 \times 1.052$$

M_S: Amount (mg) of pravastatin in taken Pravastatin 1,1,3,3-Tetramethylbutylammonium RS, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (3 in 10,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Pravastatin Sodium.

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 pravastatin 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 ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Prazepam

プラゼパム
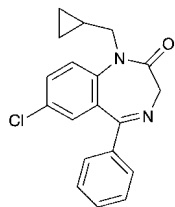
C$_{19}$H$_{17}$ClN$_2$O: 324.80
7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-
2*H*-1,4-benzodiazepin-2-one
[*2955-38-6*]

Prazepam, when dried, contains not less than 98.5% of prazepam (C$_{19}$H$_{17}$ClN$_2$O).

Description Prazepam occurs as white to light yellow crystals or crystalline powder. It is odorless.

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

Identification (1) Dissolve 0.01 g of Prazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a grayish blue fluorescence.

(2) Dissolve 0.01 g of Prazepam in 1000 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 Prazepam, 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 Flame Coloration Tests <1.04> (2) with Prazepam: a green color appears.

Melting point <2.60> 145 – 148°C

Purity (1) Chloride <1.03>—To 1.0 g of Prazepam 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) Sulfate <1.14>—To 20 mL of the filtrate 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 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Prazepam 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 Prazepam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.40 g of Prazepam in 10 mL of acetone, and use this solution as the sample solu-

tion. Pipet 1 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 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 and acetone (9:1) 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, 105°C, 2 hours).

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

Assay Weigh accurately about 0.4 g of Prazepam, 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.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 32.48 mg of C$_{19}$H$_{17}$ClN$_2$O

</div>

Containers and storage Containers—Tight containers.

Prazepam Tablets

プラゼパム錠

Prazepam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of prazepam (C$_{19}$H$_{17}$ClN$_2$O: 324.80).

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

Identification (1) To a quantity of powdered Prazepam Tablets, equivalent to 0.05 g of Prazepam, add 25 mL of acetone, shake well, and filter. Take 5 mL of the filtrate, evaporate on a water bath to dryness, and dissolve the residue in 3 mL of sulfuric acid. With this solution, proceed as directed in the Identification (1) under Prazepam.

(2) To a quantity of powdered Prazepam Tablets, equivalent to 0.02 g of Prazepam, add 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and filter. To 5 mL of the filtrate add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 50 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 241 nm and 245 nm, between 283 nm and 287 nm and between 363 nm and 367 nm, and minima between 263 nm and 267 nm and between 334 nm and 338 nm.

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of 0.1 mol/L hydrochloric acid TS as the dissolution medium, the dissolution rate in 30 minutes of Prazepam Tablets is not less than 80%.

Start the test with 1 tablet of Prazepam 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, measure exactly the subsequent *V* mL of the filtrate, add the dissolution medium to make exactly

V' mL so that each mL contains about 5 μg of prazepam ($C_{19}H_{17}ClN_2O$), and use this solution as the sample solution. Separately, weigh accurately about 5 mg of prazepam for assay, previously dried at 105°C for 2 hours, add 200 mL of the dissolution medium and dissolve with shaking, or by ultrasonication if necessary, add 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 standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of prazepam ($C_{19}H_{17}ClN_2O$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$$

M_S: Amount (mg) of prazepam for assay taken
C: Labeled amount (mg) of prazepam ($C_{19}H_{17}ClN_2O$) in 1 tablet

Assay Weigh accurately not less than 20 Prazepam Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 50 mg of prazepam ($C_{19}H_{17}ClN_2O$), add 30 mL of acetone, shake well, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with 30 mL each of acetone, combine all the supernatants liquid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), 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
= 6.496 mg of $C_{19}H_{17}ClN_2O$

Containers and storage Containers—Tight containers.

Prazosin Hydrochloride

プラゾシン塩酸塩
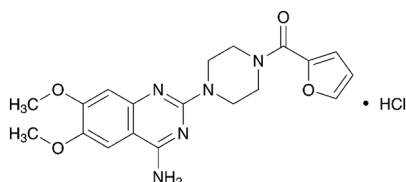
$C_{19}H_{21}N_5O_4 \cdot HCl$: 419.86
1-(4-Amino-6,7-dimethoxy-quinazolin-2-yl)-
4-(2-furoyl)piperazine monohydrochloride
[*19237-84-4*]

Prazosin Hydrochloride, when dried, contains not less than 97.0% and not more than 103.0% of prazosin hydrochloride ($C_{19}H_{21}N_5O_4 \cdot HCl$).

Description Prazosin Hydrochloride occurs as a white crystalline powder.

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

It gradually turns pale yellowish white on exposure to light.

Melting point: about 270°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Prazosin Hydrochloride 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 Prazosin 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 Prazosin 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 Prazosin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Prazosin Hydrochloride add 5 mL of water and 1 mL of ammonia TS, shake, allow to stand for 5 minutes, and filter. Render the filtrate acid with acetic acid (100): the solution responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Prazosin Hydrochloride according to 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 20 mg of Prazosin Hydrochloride 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 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 each peak other than prazosin from the sample solution is not larger than 2 times the peak area of prazosin from the standard solution, and the total area of the peaks other than prazosin from the sample solution is not larger than 5 times the peak area of prazosin 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 3.484 g of sodium 1-pentane sulfonate and 18 mL of tetramethylammonium hydroxide in 900 mL of water, adjust the pH to 5.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 1000 mL of methanol.

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

Time span of measurement: About 6 times as long as the retention time of prazosin.

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 prazosin obtained from 20 μL of this solution is equivalent to 35 to 65% of that of prazosin 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 prazosin 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 prazosin is not more than 2.0%.

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 25 mg each of Prazosin Hydrochloride and Prazosin Hydrochloride RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, and add a mixture of methanol and water (7:3) 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 prazosin in each solution.

Amount (mg) of prazosin hydrochloride ($C_{19}H_{21}N_5O_4$.HCl)
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of Prazosin Hydrochloride RS taken

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: A mixture of methanol, water, acetic acid (100) and diethylamine (3500:1500:50:1).
Flow rate: Adjust so that the retention time of prazosin 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 prazosin are not less than 5000 and not more than 2.0, respectively.
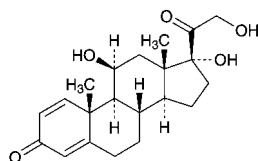System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of prazosin is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Prednisolone

プレドニゾロン

$C_{21}H_{28}O_5$: 360.44
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione
[*50-24-8*]

Prednisolone, when dried, contains not less than 97.0% and not more than 102.0% of prednisolone ($C_{21}H_{28}O_5$).

Description Prednisolone occurs as a white crystalline powder.
It is soluble in methanol and in ethanol (95), slightly soluble in ethyl acetate, and very slightly soluble in water.
Melting point: about 235°C (with decomposition).
It shows crystal polymorphism.

Identification (1) To 2 mg of Prednisolone add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.
(2) Determine the infrared absorption spectrum of Prednisolone, 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 Prednisolone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Prednisolone and Prednisolone RS in ethyl acetate, respectively, then evaporate the ethyl acetate to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +113 − +119° (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

Purity (1) Selenium—To 0.10 g of Prednisolone 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.
(2) Related substances—Dissolve 20 mg of Prednisolone in exactly 2 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of hydrocortisone and 10 mg of prednisolone acetate each in a mixture of methanol and chloroform (1:1) to make exactly 100 mL, 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 acetone, toluene and diethylamine (55:45:2) to a distance of about 15 cm, and air-dry the plate (do not dip the filter paper in the developing vessel). Spray evenly alkaline blue tetrazolium TS on the plate: the spots from the sample solution corresponding to those from the standard solutions (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), and no spots

other than the principal spot, hydrocortisone and prednisolone acetate appear from the sample 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 25 mg each of Prednisolone and Prednisolone RS, previously dried, and accurately weighed, in 50 mL of methanol, add exactly 25 mL of the internal standard solution to each, and add methanol to make 100 mL. To 1 mL each of these solutions add the mobile phase to make 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 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 prednisolone to that of the internal standard.

Amount (mg) of prednisolone ($C_{21}H_{28}O_5$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Prednisolne RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 247 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with fluorosilanized 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 (13:7).
Flow rate: Adjust so that the retention time of prednisolone is about 15 minutes.
System suitability—
System performance: Dissolve 25 mg of Prednisolone and 25 mg of hydrocortisone in 100 mL of methanol. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrocortisone and prednisolone 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 prednisolone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Prednisolone Tablets

プレドニゾロン錠

Prednisolone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44).

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

Identification (1) Weigh a quantity of powdered Prednisolone Tablets, equivalent to 0.05 g of Prednisolone, add 10 mL of chloroform, shake for 15 minutes, and filter. Evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour, and proceed as directed in the Identification (1) under Prednisolone.

(2) Determine the infrared absorption spectra of the residue obtained in (1) and Prednisolone RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the RS in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

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 Prednisolone Tablets to a volumetric flask, and shake with 10 mL of water until the tablet is disintegrated. Add 50 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, and add methanol to make exactly V' mL to provide a solution that contains about 10 μg of prednisolone ($C_{21}H_{28}O_5$) per ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 10 mL of water and 50 mL of methanol, 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. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of prednisolone ($C_{21}H_{18}O_5$)
= $M_S \times A_T/A_S \times V'/V \times 1/10$

M_S: Amount (mg) of Prednisolone RS taken

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Prednisolone Tablets is not less than 70%.

Start the test with 1 tablet of Prednisolone 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 10 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in ethanol (95) 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 the maximum wavelength at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of prednisolone ($C_{21}H_{28}O_5$)
= $M_S \times A_T/A_S \times 1/C \times 45$

M_S: Amount (mg) of Prednisolone RS taken
C: Labeled amount (mg) of prednisolone ($C_{21}H_{28}O_5$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Prednisolone Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 5 mg of prednisolone ($C_{21}H_{28}O_5$), add 1 mL of water, and shake gently. Add exactly 5 mL of the internal standard solution and 15 mL of methanol, and shake vigorously for 20 minutes. To 1 mL of this solution add the mobile phase to make 10 mL, and filter through a membrane filter with pore size of 0.45 μm. Dis-

card the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, add exactly 25 mL of the internal standard solution, and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Prednisolone with these solutions.

$$\text{Amount (mg) of prednisolone } (C_{21}H_{28}O_5)$$
$$= M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of Prednisolone RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

Containers and storage   Containers—Tight containers.

Prednisolone Acetate

プレドニゾロン酢酸エステル
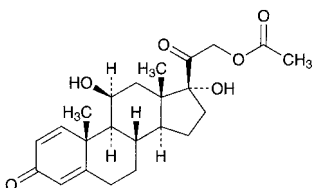
$C_{23}H_{30}O_6$: 402.48
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione
21-acetate
[*52-21-1*]

Prednisolone Acetate, when dried, contains not less than 96.0% and not more than 102.0% of prednisolone acetate ($C_{23}H_{30}O_6$).

Description   Prednisolone Acetate occurs as a white crystalline powder.
   It is slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.
   Melting point: about 235°C (with decomposition).
   It shows crystal polymorphism.

Identification   (1)   To 2 mg of Prednisolone Acetate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.
   (2)   Determine the infrared absorption spectra of Prednisolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum in a range between 4000 cm^{-1} and 650 cm^{-1} with the Infrared Reference Spectrum or the spectrum of previously dried Prednisolone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the RS in ethanol (99.5), respectively, evaporate to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +128 – +137° (after drying, 70 mg, methanol, 20 mL, 100 mm).

Purity   Related substanes—Dissolve 0.20 g of Prednisolone Acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution.

Separately, dissolve 20 mg each of prednisolone, cortisone acetate and hydrocortisone acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1). Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) 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 a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (wavelength: 254 mm): the spots from the sample solution corresponding to those from the standard solution are not more intense than the spots from the standard solution, and any spot from the sample solution other than the principal spot and the spots from prednisolone, cortisone acetate and hydrocortisone acetate does not appear.

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 10 mg each of Prednisolone Acetate and Prednisolone Acetate RS, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 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 text 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 prednisolone acetate to that of the internal standard.

$$\text{Amount (mg) of prednisolone acetate } (C_{23}H_{30}O_6)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Prednisolone Acetate RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 1000).
Operating conditions—
   Detector: An ultraviolet absorption photometer (wavelength: 254 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 (5 μm in particle diameter).
   Column temperature: A constant temperature of about 25°C.
   Mobile phase: A mixture of water and acetonitrile (3:2).
   Flow rate: Adjust so that the retention time of prednisolone acetate 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, prednisolone acetate 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 ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Prednisolone Sodium Phosphate

プレドニゾロンリン酸エステルナトリウム
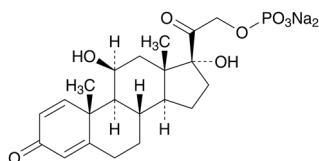
$C_{21}H_{27}Na_2O_8P$: 484.39
11β,17,21-Trihydroxypregna-1,4-diene 3,20-dione
21-(disodium phosphate)
[*125-02-0*]

Prednisolone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of prednisolone sodium phosphate ($C_{21}H_{27}Na_2O_8P$), calculated on the anhydrous basis.

Description Prednisolone Sodium Phosphate occurs as a white to pale yellow powder.

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

It is hygroscopic.

Identification (1) Moisten 1.0 g of Prednisolone Sodium Phosphate with a small amount of sulfuric acid, and gradually heat to incinerate. 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 phosphate.

(2) Dissolve 2 mg of Prednisolone Sodium Phosphate in 2 mL of sulfuric acid, and allow to stand for 2 minutes: a deep red color, without fluorescence, develops.

(3) Determine the absorption spectrum of a solution of Prednisolone Sodium Phosphate (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 Prednisolone Sodium Phosphate 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.

(5) The solution obtained in (1) responds to the Qualitative Tests <*1.09*> for sodium salt.

Optical rotation <*2.49*> [α]$_D^{20}$: +96 – +103° (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 Prednisolone Sodium Phosphate in 100 mL of water: the pH of the solution is between 7.5 and 9.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Prednisolone Sodium Phosphate in 10 mL of water: the solution is clear and not more colored than the following control solution.

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.4 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make 10 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 100 mL.

(2) Heavy metals <*1.07*>—Proceed with 0.5 g of Pred-

nisolone Sodium Phosphate according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(3) Free phosphoric acid—Weigh accurately about 0.25 g of Prednisolone 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 Phosphoric Acid Standard Solution, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphtol-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, of each solution from the sample solution and standard solution at 740 nm: the content of free phosphoric acid is not more than 1.0%.

$$\text{Content (\%) of free phosphoric acid (H}_3\text{PO}_4)$$
$$= 1/M \times A_T/A_S \times 258.0$$

M: Amount (mg) of Prednisolone Sodium Phosphate taken, calculated on the anhydrous basis

(4) Related substances—Dissolve 10 mg of Prednisolone Sodium Phosphate 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. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than prednisolone phosphate from the sample solution is not larger than 1.5 times the peak area of prednisolone phosphate from the standard solution, and the total area of the peaks other than prednisolone phosphate from the sample solution is not larger than 2.5 times the peak area of prednisolone phosphate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 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: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid. To 1000 mL of this solution add 250 mL of acetonitrile.

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

Time span of measurement: About 4 times as long as the retention time of prednisolone phosphate.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of prednisolone phosphate obtained from 20 μL of this solution is equivalent to 7 to 13% of that of prednisolone phosphate 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 prednisolone phosphate 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 prednisolone phosphate is not more than 2.0%.

Water <2.48>   Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Assay   Weigh accurately about 0.1 g of Prednisolone Sodium Phosphate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add 1 mL of alkaline phosphatase TS, and allow to stand for 2 hours with occasional shaking. To this solution add exactly 20 mL of 1-octanol, and shake vigorously. Centrifuge this solution, pipet 10 mL of the 1-octanol layer, add 1-octanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone RS, previously dried at 105°C for 3 hours, and dissolve in 1-octanol to make exactly 100 mL. Pipet 6 mL of this solution, add a solution prepared by adding 1 mL of alkaline phosphatase TS to 2 mL water and being allowed to stand for 2 hours with occasional gentle shaking, add exactly 14 mL of 1-octanol, and shake vigorously. Proceed in the same manner as the sample solution to make the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 1-octanol as the blank, and determine the absorbances, A_T and A_S, at 245 nm.
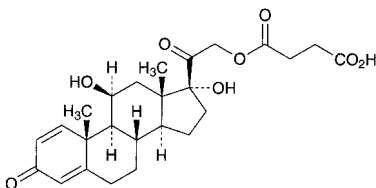
Amount (mg) of prednisolone sodium phosphate
($C_{21}H_{27}Na_2O_8P$)
    $= M_S \times A_T/A_S \times 3 \times 1.344$

M_S: Amount (mg) of Prednisolone RS taken

Containers and storage   Containers—Tight containers.

Prednisolone Succinate

プレドニゾロンコハク酸エステル

$C_{25}H_{32}O_8$: 460.52
11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(hydrogen succinate)
[*2920-86-7*]

Prednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of prednisolone succinate ($C_{25}H_{32}O_8$).

Description   Prednisolone Succinate occurs as a white, fine, crystalline powder. It is odorless.

It is freely soluble in methanol, soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

Melting point: about 205°C (with decomposition).

Identification   **(1)**   To 2 mg of Prednisolone Succinate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2)   Determine the infrared absorption spectrum of Prednisolone Succinate 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 Prednisolone Succinate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +114 – +120° (after drying, 67 mg, methanol, 10 mL, 100 mm).

Purity   Related substances—Dissolve 0.10 g of Prednisolone Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 30 mg of prednisolone in methanol to make exactly 10 mL. Pipet 1 mL of the 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 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 ethanol (95) (2:1) 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, in vacuum, phosphorus (V) oxide, 60°C, 6 hours).

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

Assay   Weigh accurately about 10 mg each of Prednisolone Succinate and Prednisolone Succinate RS, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 50 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 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.
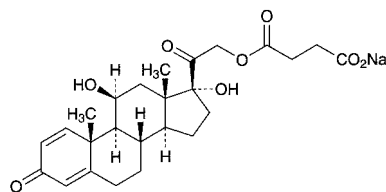
Amount (mg) of prednisolone succinate ($C_{25}H_{32}O_8$)
    $= M_S \times A_T/A_S$

M_S: Amount (mg) of Prednisolone Succinate RS taken

Containers and storage   Containers—Tight containers.

Prednisolone Sodium Succinate for Injection

注射用プレドニゾロンコハク酸エステルナトリウム

$C_{25}H_{31}NaO_8$: 482.50
Monosodium 11β,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-succinate
[*1715-33-9*]

Prednisolone Sodium Succinate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 72.4% and not more

than 83.2% of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$), and the equivalent of not less than 90.0% and not more than 110.0% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44).

The amount should be stated as the amount of prednisolone ($C_{21}H_{28}O_5$).

Method of preparation Prepare as directed under Injections, with Prednisolone Succinate and Dried Sodium Carbonate or Sodium Hydroxide.

It contains a suitable buffer agent.

Description Prednisolone Sodium Succinate for Injection occurs as a white powder or porous, friable mass.

It is freely soluble in water.

It is hygroscopic.

Identification (1) To 2 mg of Prednisolone Sodium Succinate for Injection add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Prednisolone Sodium Succinate for Injection in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) Dissolve 0.1 g of Prednisolone Sodium Succinate for Injection in 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and filter. Add 1 mL of dilute hydrochloric acid to the filtrate, shake, and filter if necessary. Adjust the solution with diluted ammonia TS (1 in 10) to a pH of about 6, and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Prednisolone Sodium Succinate for Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 1.0 g of Prednisolone Sodium Succinate for Injection in 40 mL of water: the pH of the solution is between 6.5 and 7.2.

Purity Clarity and color of solution—Dissolve 0.25 g of Prednisolone Sodium Succinate for Injection in 10 mL of water: the solution is clear and colorless.

Loss on drying <2.41> Not more than 2.0% (0.15 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 2.4 EU/mg of prednisolone ($C_{21}H_{28}O_5$).

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 quantity of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.1 g of prednisolone ($C_{21}H_{28}O_5$), and dissolve the contents in a suitable amount of diluted methanol (1 in 2), and transfer to a 100-mL volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask, and add diluted methanol (1 in 2) to make volume. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate RS, previously dried in a desiccator for 6 hours (in vacuum, phosphorus (V) oxide, 60°C), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography according <2.01> to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of prednisolone succinate to that of the internal standard.

Amount (mg) of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$)
$$= M_S \times Q_T/Q_S \times 5 \times 1.048$$

Amount (mg) of prednisolone ($C_{21}H_{28}O_5$)
$$= M_S \times Q_T/Q_S \times 5 \times 0.783$$

M_S: Amount (mg) of Prednisolone Succinate RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 25,000).
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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 0.32 g of tetra *n*-butylammonium bromide, 3.22 g of disodium hydrogen phosphate dodacahydrate and 6.94 g of potassium dihydrogen phosphate in 1000 mL of water. To 840 mL of this solution add 1160 mL of methanol.

Flow rate: Adjust so that the retention time of prednisolone succinate 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, prednisolone succinate 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 prednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Primidone

プリミドン
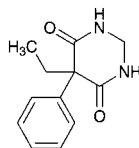
C$_{12}$H$_{14}$N$_2$O$_2$: 218.25
5-Ethyl-5-phenyl-2,3-dihyropyrimidine-4,6(1*H*,5*H*)-dione
[*125-33-7*]

Primidone, when dried, contains not less than 98.5% of primidone (C$_{12}$H$_{14}$N$_2$O$_2$).

Description Primidone occurs as a white, crystalline powder or granules. It is odorless and has a slightly bitter taste.

It is soluble in *N,N*-dimethylformamide, sparingly soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification **(1)** Heat 0.5 g of Primidone with 5 mL of diluted sulfuric acid (1 in 2): the odor of formaldehyde is perceptible.

(2) Mix 0.2 g of Primidone with 0.2 g of anhydrous sodium carbonate, and heat: the gas evolved changes moistened red litmus paper to blue.

Melting point *<2.60>*   279 – 284°C

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Primidone in 10 mL of *N,N*-dimethylformamide: the solution is clear and colorless.

(2) Heavy metals *<1.07>*—Proceed with 2.0 g of Primidone 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) 2-Ethyl-2-phenylmalonediamide—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add exactly 2 mL of the internal standard solution, then add 1 mL of bis-trimethyl silyl acetamide, shake well, and heat at 100°C for 5 minutes. Cool, add pyridine to make 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 2-ethyl-2-phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, proceed in the same manner as Primidone, and use this solution as the standard solution. Perform the test with 2 μL 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 2-ethyl-2-phenylmalonediamide to that of the internal standard: Q_T is not more than Q_S.

Internal standard solution—A solution of stearylalcohol in pyridine (1 in 2000).

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 150 cm in length, packed with siliceous earth for gas chromatography (125 to 150 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 3%.
Column temperature: A constant temperature of about 195°C.
Carrier gas: Nitrogen.
Flow rate: Adjust so that the retention time of stearylalcohol is about 10 minutes.

System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating condition, 2-ethyl-2-phenylmalonediamide 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 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5%.

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

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

Assay Weigh accurately about 20 mg each of Primidone and Primidone RS, previously dried, dissolve each in 20 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbance, A_1, of the sample solution and standard solution at the wavelength of maximum absorption at about 257 nm, and the absorbances, A_2 and A_3, at the wavelength of minimum absorption at about 254 nm and at about 261 nm, as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using ethanol (95) as the blank.

$$\text{Amount (mg) of primidone (C}_{12}\text{H}_{14}\text{N}_2\text{O}_2)$$
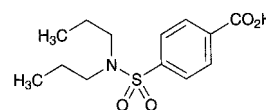$$= M_S \times (2A_1 - A_2 - A_3)_T/(2A_1 - A_2 - A_3)_S$$

M_S: Amount (mg) of Primidone RS taken

where, $(2A_1 - A_2 - A_3)_T$ is the value from the sample solution, and $(2A_1 - A_2 - A_3)_S$ is from the standard solution.

Containers and storage Containers—Tight containers.

Probenecid

プロベネシド

C$_{13}$H$_{19}$NO$_4$S: 285.36
4-(Dipropylaminosulfonyl)benzoic acid
[*57-66-9*]

Probenecid, when dried, contains not less than 98.0% of probenecid (C$_{13}$H$_{19}$NO$_4$S).

Description Probenecid occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste, followed by unpleasant bitter.

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

It dissolves in sodium hydroxide TS and in ammonia TS.
Melting point: 198 – 200°C

Identification **(1)** Heat Probenecid strongly: the odor of sulfur dioxide is perceptible.

(2) Determine the absorption spectrum of a solution of Probenecid in ethanol (99.5) (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 Probenecid RS prepared in the same manner

as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Acidity—To 2.0 g of Probenecid add 100 mL of water, heat on a water bath with occasional shaking for 30 minutes, cool, and filter. To the filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Chloride <1.03>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of nitric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate 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>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of hydrochloric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Probenecid 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) Arsenic <1.11>—Prepare the test solution with 1.0 g of Probenecid according to Method 3, and perform the test (not more than 2 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 0.5 g of Probenecid, previously dried, and dissolve in 50 mL of neutralized ethanol. Titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

$$\text{Each mL of 0.1 mol/L sodium hydroxide VS}$$
$$= 28.54 \text{ mg of } C_{13}H_{19}NO_4S$$

Containers and storage Containers—Well-closed containers.

Probenecid Tablets

プロベネシド錠

Probenecid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of probenecid ($C_{13}H_{19}NO_4S$: 285.36).

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

Identification **(1)** Weigh a quantity of powdered Probenecid Tablets, equivalent to 0.5 g of Probenecid, add 50 mL of ethanol (95) and 1 mL of 1 mol/L hydrochloric acid TS, shake, and filter. Evaporate the filtrate on a water bath to about 20 mL. After cooling, collect produced crystals, recrystallize with 50 mL of dilute ethanol, and dry at 105°C for 4 hours: it melts <2.60> between 196°C and 200°C. With the crystals so obtained, proceed as directed in the Identification (1) under Probenecid.

(2) Determine the absorption spectrum of a solution of the dried crystals obtained in (1) in ethanol (99.5) (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 Probenecid RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

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 Probenecid Tablets add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, treat with ultrasonic waves with occasional shaking to disintegrate the tablet completely, and add ethanol (99.5) to make exactly 100 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add ethanol (99.5) to make exactly V mL so that each mL contains about 15 μg of probenecid ($C_{13}H_{19}NO_4S$), and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probenecid RS, previously dried at 105°C for 4 hours, dissolve in 15 mL of water, 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of this solution, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (99.5) 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 Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by adding ethanol (99.5) to 1 mL of 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, as the blank, and determine the absorbances, A_T and A_S, at 248 nm.

$$\text{Amount (mg) of probenecid } (C_{13}H_{19}NO_4S)$$
$$= M_S \times A_T/A_S \times V/25$$

M_S: Amount (mg) of Probenecid RS taken

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Probenecid Tablets is not less than 80%.

Start the test with 1 tablet of Probenecid 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 contains about 14 μg of probenecid ($C_{13}H_{19}NO_4S$), and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Probenecid RS, previously dried at 105°C for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 1 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 244 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Dissolution rate (\%) with respect to the labeled amount}$$
$$\text{of probenecid } (C_{13}H_{19}NO_4S)$$
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of Probenecid RS taken
C: Labeled amount (mg) of probenecid ($C_{13}H_{19}NO_4S$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Probenecid Tablets. Weigh accurately a portion of the pow-

der, equivalent to about 0.25 g of probenecid ($C_{13}H_{19}NO_4S$), add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, shake, add 30 mL of ethanol (99.5), disperse the particles with the aid of ultrasonic waves, and add ethanol (99.5) to make exactly 100 mL. Centrifuge the solution, pipet 3 mL of the supernatant liquid, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probenecid RS, previously dried at 105°C for 4 hours, add 15 mL of water and 1 mL of 1 mol/L hydrochloric acid TS, then add ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of this solution, add 1 mL of 1 mol/L hydrochloric acid TS, and add ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of the solution, add ethanol (99.5) 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 248 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by mixing 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient ethanol (99.5) to make exactly 50 mL, as the blank.
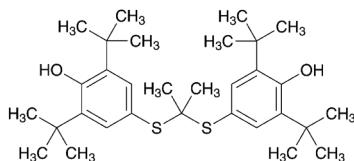
$$\text{Amount (mg) of probenecid } (C_{13}H_{19}NO_4S) = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of Probenecid RS taken

Containers and storage Containers—Well-closed containers.

Probucol

プロブコール

$C_{31}H_{48}O_2S_2$: 516.84
4,4′-[Propan-2,2-diylbis(sulfandiyl)]bis[2,6-bis(1,1-dimethylethyl)phenol]
[23288-49-5]

Probucol, when dried, contains not less than 98.5% and not more than 101.0% of probucol ($C_{31}H_{48}O_2S_2$).

Description Probucol occurs as a white crystalline powder.

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

It gradually turns light yellow on exposure to light.

Identification (1) Determine the absorption spectrum of a solution of Probucol 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 Probucol 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 Probucol 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 Probucol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 125 – 128°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Probucol 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 0.40 g of Probucol in 5 mL of ethanol (99.5), add the mobile phase to make 20 mL, 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 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 0.9 to probucol from the sample solution is not larger than the peak area of probucol from the standard solution, and the area of peak having the relative retention time of about 1.9 to probucol from the sample solution is not larger than 25 times the peak area of probucol from the standard solution, and the area of each peak other than probucol and the peaks mentioned above from the sample solution is not larger than 5 times the peak area of probucol from the standard solution. Furthermore, the total area of the peaks other than probucol from the sample solution is not larger than 50 times the peak area of probucol from the standard solution. For the areas of the peaks, having the relative retention times of about 0.9 and about 1.9 to probucol, multiply their relative response factors, 1.2 and 1.4, 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 3 times as long as the retention time of probucol, beginning after the solvent peak, excluding the peak having the relative retention time of about 0.5 to probucol.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of probucol obtained from 5 μL of this solution is equivalent to 14 to 26% of that of probucol obtained from 5 μL of the standard solution.

System performance: To 1 mL of the sample solution add the mobile phase to make 50 mL. To 1 mL of this solution add 1 mL of a solution of phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) in the mobile phase (1 in 1000), 5 mL of ethanol (99.5), and the mobile phase to make 20 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) and probucol 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of probucol is not more than 5%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 80°C, 1 hour).

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

Assay Weigh accurately about 60 mg each of Probucol and Probucol RS, previously dried, dissolve each in 5 mL of tetrahydrofuran, and add the mobile phase to make exactly

50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with $10\,\mu$L 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 probucol to that of the internal standard.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2)$$
$$= M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Probucol RS taken

Internal standard solution—Dissolve 0.2 g of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in 1 mL of tetrahydrofuran, and add the mobile phase to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 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 ($5\,\mu$m in particle diameter).

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

Mobile phase: A mixture of acetonitrile and water (93:7).

Flow rate: Adjust so that the retention time of probucol 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, the internal standard and probucol 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 $10\,\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

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

Probucol Fine Granules

プロブコール細粒

Probucol Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of probucol ($C_{31}H_{48}O_2S_2$: 516.84).

Method of preparation Prepare as directed under Granules, with Probucol.

Identification To an amount of powdered Probucol Fine Granules, equivalent to 50 mg of Probucol, add 100 mL of methanol, shake, and filter. To 2 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 a maximum between 240 nm and 244 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: the granules in single-dose packages meet the requirement of the Content uniformity test.

To the total amount of the content of 1 package of Probucol Fine Granules add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centri-

fuge, pipet V mL of the supernatant liquid, equivalent to about 5 mg of probucol ($C_{31}H_{48}O_2S_2$), add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2)$$
$$= M_S \times Q_T/Q_S \times 10/V$$

M_S: Amount (mg) of Probucol RS taken

Internal standard solution—A solution of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Assay Weigh accurately an amount of powdered Probucol Fine Granules, equivalent to about 0.25 g of probucol ($C_{31}H_{48}O_2S_2$), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with $10\,\mu$L 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 probucol to that of the internal standard.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2)$$
$$= M_S \times Q_T/Q_S \times 5$$

M_S: Amount (mg) of Probucol RS taken

Internal standard solution—A solution of bis(cis-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Operating conditions—

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

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

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 probucol 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 $10\,\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Probucol Tablets

プロブコール錠

Probucol Tablets contain not less than 95.0% and not more than 105.0% of probucol ($C_{31}H_{48}O_2S_2$: 516.84).

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

Identification   To an amount of powdered Probucol Tablets, equivalent to 50 mg of Probucol, add 100 mL of methanol, shake, and filter. To 2 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 a maximum between 240 nm and 244 nm.

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

Shake 1 tablet of Probucol Tablets with a suitable amount of methanol until the tablet is disintegrated, and add methanol to make exactly *V* mL so that each mL of the solution contains about 2.5 mg of probucol ($C_{31}H_{48}O_2S_2$). Centrifuge the solution, pipet 2 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. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2)$$
$$= M_S \times Q_T/Q_S \times V/20$$

M_S: Amount (mg) of Probucol RS taken

Internal standard solution—A solution of bis(*cis*-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).

Disintegration <6.09>   It meets the requirement.

Assay   Weigh accurately the mass of 20 Probucol Tablets, and powder the tablets. Weigh accurately a portion of the powder, equivalent to about 0.25 g of probucol ($C_{31}H_{48}O_2S_2$), add 70 mL of methanol, shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Probucol RS, previously dried under reduced pressure at 80°C for 1 hour, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, 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 probucol to that of the internal standard.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2)$$
$$= M_S \times Q_T/Q_S \times 5$$

M_S: Amount (mg) of Probucol RS taken

Internal standard solution—A solution of bis(*cis*-3,3,5-trimethylcyclohexyl) phthalate in methanol (1 in 250).
Operating conditions—

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

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 10 µL of the standard solution under the above operating conditions, the internal standard and probucol 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 probucol to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Well-closed containers.

Procainamide Hydrochloride

プロカインアミド塩酸塩
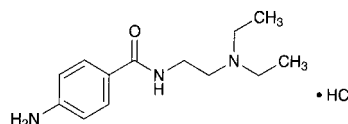
$C_{13}H_{21}N_3O.HCl$: 271.79
4-Amino-*N*-(2-diethylaminoethyl)benzamide monohydrochloride
[*614-39-1*]

Procainamide Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$).

Description   Procainamide Hydrochloride occurs as a white to light yellow crystalline powder.
It is very soluble in water and soluble in ethanol (99.5).
It is hygroscopic.

Identification   (1)   Determine the infrared absorption spectrum of Procainamide 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.

(2)   A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54>   Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

Melting point <2.60>   165 – 169°C

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

(2)   Heavy metals <1.07>—Proceed with 2.0 g of Procainamide 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).

(3)   Arsenic <1.11>—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4)   Related substances—Dissolve 50 mg of Procainamide Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample so-

lution, and add the mobile phase to make exactly 50 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 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 the peaks other than procainamide from the sample solution is not larger than the peak area of procainamide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 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 0.02 mol/L phosphate buffer solution (pH 3.0) and methanol (9:1).

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

Time span of measurement: About 2 times as long as the retention time of procainamide.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of procainamide 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: 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 procainamide 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 procainamide is not more than 2.0%.

Loss on drying <2.41> Not more than 0.3% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Assay Weigh accurately about 0.5 g of Procainamide 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
= 27.18 mg of $C_{13}H_{21}N_3O.HCl$

Containers and storage Containers—Tight containers.

Procainamide Hydrochloride Injection

プロカインアミド塩酸塩注射液

Procainamide Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$: 271.79).

Method of preparation Prepare as directed under Injections, with Procainamide Hydrochloride.

Description Procainamide Hydrochloride Injection is a clear, colorless or light yellow liquid.

pH: 4.0 – 6.0

Identification (1) To a volume of Procainamide Hydrochloride Injection, equivalent to 10 mg of Procainamide Hydrochloride, add 1 mL of dilute hydrochloric acid and water to make 5 mL: the solution responds to the Qualitative Tests <1.09> (1) for primary aromatic amines.

(2) To a volume of Procainamide Hydrochloride Injection, equivalent to 0.1 g of Procainamide Hydrochloride, add water to make 100 mL. To 1 mL of this solution add water to make 100 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.

(3) Procainamide Hydrochloride Injection responds to the Qualitative Tests <1.09> (2) for chloride.

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 Dilute an accurately measured volume of Procainamide Hydrochloride Injection, equivalent to about 0.5 g of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$), with 5 mL of hydrochloric acid and water to 50 mL, add 10 mL of potassium bromide solution (3 → 10), cool to 15°C or lower, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration method or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.18 mg of $C_{13}H_{21}N_3O.HCl$

Containers and storage Containers—Hermetic containers.

Procainamide Hydrochloride Tablets

プロカインアミド塩酸塩錠

Procainamide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$: 271.79).

Method of preparation Prepare as directed under Tablets, with Procainamide Hydrochloride.

Identification To a quantity of powdered Procainamide Hydrochloride Tablets, equivalent to 1.5 g of Procainamide Hydrochloride, add 30 mL of water, shake well, filter, and use the filtrate as the sample solution. To 0.2 mL of the sample solution add 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

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 Procainamide Hydrochloride Tablets add $3V/5$ mL of 0.02 mol/L phosphate buffer solution (pH 3.0) treat with ultrasonic waves to disintegrate the tablet completely, add 0.02 mol/L phosphate buffer solution (pH 3.0) to make exactly V mL so that each mL contains about 2.5 mg of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$), and shake for 5 minutes. Centrifuge this solution, pipet 1 mL of the supernatant liquid, add 0.02 mol/L phosphate buffer solution (pH 3.0) to make exactly 250 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of procainamide hydrochloride
($C_{13}H_{21}N_3O.HCl$)
$$= M_S \times A_T/A_S \times V/20$$

M_S: Amount (mg) of procainamide 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 Procainamide Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Procainamide Hydrochloride 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 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 7 μg of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test 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 278 nm.

Dissolution rate (%) with respect to the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$$

M_S: Amount (mg) of procainamide hydrochloride for assay taken
C: Labeled amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$) in 1 tablet

Assay   To 10 Procainamide Hydrochloride Tablets add about 300 mL of 0.02 mol/L phosphate buffer solution (pH 3.0) and treat with ultrasonic waves to disintegrate the tablets completely. To this solution add 0.02 mol/L phosphate buffer solution (pH 3.0) to make exactly 500 mL, and stir for 5 minutes. Centrifuge this solution, pipet V mL of the supernatant liquid, and add 0.02 mol/L phosphate buffer solution (pH 3.0) to make exactly V' mL so that each mL contains about 10 μg of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$). 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 50 mg of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, dissolve in 0.02 mol/L phosphate buffer solution (pH 3.0) to make exactly 100 mL. Pipet 2 mL of this solution, add 0.02 mol/L phosphate buffer solution (pH 3.0) 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 the peak areas, A_T and A_S, of procainamide in each solution.

Amount (mg) of procainamide hydrochloride
($C_{13}H_{21}N_3O.HCl$)
$$= M_S \times A_T/A_S \times V'/V \times 1/10$$

M_S: Amount (mg) of procainamide hydrochloride for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 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 0.02 mol/L phosphate buffer solution (pH 3.0) and methanol (9:1).

Flow rate: Adjust so that the retention time of procainamide is about 9 minutes.
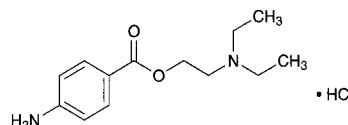
System suitability—

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 procainamide 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 procainamide is not more than 1.0%.

Containers and storage   Containers—Tight containers.

Procaine Hydrochloride

プロカイン塩酸塩

$C_{13}H_{20}N_2O_2.HCl$: 272.77
2-(Diethylamino)ethyl 4-aminobenzoate monohydrochloride
[*51-05-8*]

Procaine Hydrochloride, when dried, contains not less than 99.0% of procaine hydrochloride ($C_{13}H_{20}N_2O_2.HCl$).

Description   Procaine Hydrochloride occurs as white crystals or crystalline powder.

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

Identification   **(1)**   Determine the absorption spectrum of a solution of Procaine 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 Procaine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Refer-

ence Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Procaine Hydrochloride (1 in 10) responds to the Qualitative Tests *<1.09>* for chloride.

pH *<2.54>* The pH of a solution prepared by dissoluing 1.0 g of Procaine Hydrochloride in 20 mL of water is between 5.0 and 6.0.

Melting point *<2.60>* 155 – 158°C

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

(2) Heavy metals *<1.07>*—Proceed with 1.0 g of Procaine 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) Related substances—To 1.0 g of Procaine Hydrochloride add 5 mL of ethanol (95), dissolve by mixing well, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-aminobenzoic acid in ethanol (95) to make exactly 20 mL, then pipet 1 mL of this solution, add 4 mL of ethanol (95) and 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 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 dibutyl ether, *n*-hexane and acetic acid (100) (20:4:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate more at 105°C for 10 minutes, 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. The principal spot from the sample solution stays at the origin.

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 0.4 g of Procaine Hydrochloride, previously dried, dissolve in 5 mL of hydrochloric acid and 60 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool to below 15°C, and titrate *<2.50>* with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

<div align="center">

Each mL of 0.1 mol/L sodium nitrite VS
= 27.28 mg of $C_{13}H_{20}N_2O_2 \cdot HCl$

</div>

Containers and storage Containers—Well-closed containers.

Procaine Hydrochloride Injection

プロカイン塩酸塩注射液

Procaine Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$: 272.77).

Method of preparation Prepare as directed under Injections, with Procaine Hydrochloride.

Description Procaine Hydrochloride Injection is a clear, colorless liquid.

Identification (1) To a volume of Procaine Hydrochloride Injection, equivalent to 0.01 g of Procaine Hydrochloride, add water to make 1000 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 289 nm and 293 nm.

(2) Procaine Hydrochloride Injection responds to the Qualitative Tests *<1.09>* (2) for chloride.

pH *<2.54>* 3.3 – 6.0

Bacterial endotoxins *<4.01>* Less than 0.02 EU/unit. Apply to the preparations intended for intraspinal administration.

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 Procaine Hydrochloride Injection, equivalent to about 20 mg of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 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 50 mg of procaine hydrochloride for assay, previously dried in a desiccator (silica gel) 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 and 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 procaine hydrochloride to that of the internal standard.

<div align="center">

Amount (mg) of procaine hydrochloride
($C_{13}H_{20}N_2O_2 \cdot HCl$)
= $M_S \times Q_T/Q_S \times 2/5$

</div>

M_S: Amount (mg) of procaine hydrochloride for assay taken

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).
Operating conditions—
  Detector: An ultraviolet absorption photometer (wavelength: 254 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 40°C.
  Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid, and add an amount of sodium 1-pentane sulfonate to make a solution so that containing 0.1%. To 800 mL of this solution add 200 mL of methanol.
  Flow rate: Adjust so that the retention time of procaine 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, procaine 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 ratios of the peak area of procaine to that of the internal standard is not more than 1.0%.

Containers and storage   Containers—Hermetic containers.

Procarbazine Hydrochloride

プロカルバジン塩酸塩
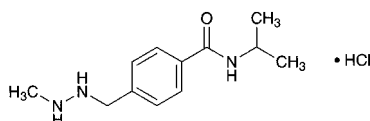
C$_{12}$H$_{19}$N$_3$O.HCl: 257.76
N-(1-Methylethyl)-
4-[(2-methylhydrazino)methyl]benzamide
monohydrochloride
[*366-70-1*]

Procarbazine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of procarbazine hydrochloride (C$_{12}$H$_{19}$N$_3$O.HCl).

Description   Procarbazine Hydrochloride occurs as white to light yellowish white crystals or crystalline powder.

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

It dissolves in dilute hydrochloric acid.

Melting point: about 223°C (with decomposition).

Identification   **(1)**   Dissolve 0.01 g of Procarbazine Hydrochloride in 1 mL of diluted copper (II) sulfate TS (1 in 10), and add 4 drops of sodium hydroxide TS: a green precipitate is formed immediately, and the color changes from green through yellow to orange.

(2)   Determine the absorption spectrum of a solution of Procarbazine 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.

(3)   Determine the infrared absorption spectrum of Procarbazine 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 Procarbazine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54>   Dissolve 0.10 g of Procarbazine Hydrochloride in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

Purity   **(1)**   Heavy metals <1.07>—Proceed with 1.0 g of Procarbazine 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).

(2)   Related substances—Dissolve 50 mg of Procarbazine Hydrochloride in 5.0 mL of a solution of L-cysteine hydro-

chloride monohydrate in diluted methanol (7 in 10) (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200) 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>. Immerse slowly, by inclining, a plate of silica gel with fluorescent indicator for thin-layer chromatography in a solution of L-cysteine hydrochloride monohydrate in diluted methanol (7 in 10) (1 in 200), allow to stand for 1 minute, lift the plate from the solution, dry it in cold wind for 10 minutes, then dry in warm wind for 5 minutes, and then dry at 60°C for 5 minutes. After cooling, spot 5 μL each of the sample solution and standard solution on the plate. Develop the plate with a mixture of methanol and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 1 spot other than the principal spot and the spot of the starting point from the sample solution appears, and is 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 0.15 g of Procarbazine Hydrochloride, previously dried, place in a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, and cool to room temperature. To this solution add 5 mL of chloroform, and titrate <2.50>, while shaking, with 0.05 mol/L potassium iodate VS until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the purple color disappeared.

Each mL of 0.05 mol/L potassium iodate VS
= 8.592 mg of C$_{12}$H$_{19}$N$_3$O.HCl

Containers and storage   Containers—Tight containers.

Procaterol Hydrochloride Hydrate

プロカテロール塩酸塩水和物

and enantiomer
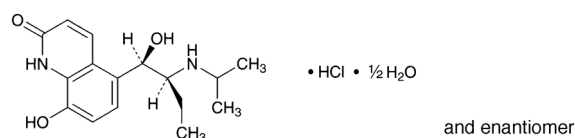
C$_{16}$H$_{22}$N$_2$O$_3$.HCl. ½H$_2$O: 335.83
8-Hydroxy-5-{(1*RS*,2*SR*)-1-hydroxy-
2-[(1-methylethyl)amino]butyl]}quinolin-2(1*H*)-one
monohydrochloride hemihydrate
[*62929-91-3*, anhydride]

Procaterol Hydrochloride Hydrate contains not less than 98.5% of procaterol hydrochloride (C$_{16}$H$_{22}$N$_2$O$_3$.HCl: 326.82), calculated on the anhydrous basis.

Description   Procaterol Hydrochloride Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is soluble in water, in formic acid and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Procaterol Hydrochloride

Hydrate in 100 mL of water is between 4.0 and 5.0.

It is gradually colored by light.

The solution of Procaterol Hydrochloride Hydrate (1 in 20) shows no optical rotation.

Melting point: about 195°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Procaterol Hydrochloride Hydrate (7 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 Procaterol 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 Procaterol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Procaterol Hydrochloride Hydrate in 30 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3.0 mL of Iron (III) Chloride CS add water to make 50 mL.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Procaterol 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).

(3) Related substances—Dissolve 0.10 g of Procaterol Hydrochloride Hydrate in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) 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 these solutions by the automatic integration method: the total area of the peaks other than procaterol from the sample solution is not larger than the peak area of procaterol from the standard solution.

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and about 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 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water. To 760 mL of this solution add 230 mL of methanol and 10 mL of acetic acid (100).

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

Selection of column: Dissolve 20 mg each of Procaterol Hydrochloride Hydrate and threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2). To 15 mL of this solution add diluted methanol (1 in 2) to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of procaterol and threoprocaterol in this order with the resolution of these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of procaterol obtained from 2 μL of the standard solution is not less than 10 mm.

Time span of measurement: 2.5 times as long as the retention time of procaterol, beginning after the solvent peak.

Water <2.48> 2.5 – 3.3% (0.5 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.25 g of Procaterol Hydrochloride Hydrate, add 2 mL of formic acid, dissolve by warming, and add exactly 15 mL of 0.1 mol/L perchloric acid VS. Add 1 mL of acetic anhydride, heat on a water bath for 30 minutes, cool, add 60 mL of acetic anhydride, and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.
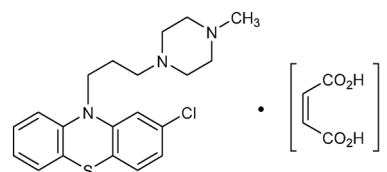
$$\text{Each mL of 0.1 mol/L perchloric acid VS}$$
$$= 32.68 \text{ mg of } C_{16}H_{22}N_2O_3 \cdot HCl$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Prochlorperazine Maleate

プロクロルペラジンマレイン酸塩

$C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$: 606.09
2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine dimaleate
[*84-02-6*]

Prochlorperazine Maleate, when dried, contains not less than 98.0% of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$).

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

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

It gradually acquires a red tint by light.

Melting point: 195 – 203°C (with decomposition).

Identification **(1)** Dissolve 5 mg of Prochlorperazine Maleate in 5 mL of sulfuric acid: a red color develops, which darkens slowly on standing. Warm a half of the solution: the color changes to red-purple. To the remainder add 1 drop of potassium dichromate TS: a green-brown color develops, which changes to brown on standing.

(2) Boil 0.5 g of Prochlorperazine Maleate with 10 mL of hydrobromic acid under a reflux condenser for 10 minutes. After cooling, add 100 mL of water, and filter through glass filter (G4). Wash the residue with three 10-mL portions of water, and dry at 105°C for 1 hour: it melts <2.60> between 195°C and 198°C (with decomposition).

(3) Dissolve 0.2 g of Prochlorperazine Maleate in 5 mL of a solution of sodium hydroxide (1 in 10), and extract with three 3-mL portions of diethyl ether [reserve the aqueous layer, and use for test (4)]. Evaporate the combined diethyl ether extracts on a water bath to dryness, dissolve the residue

in 10 mL of methanol by warming, and pour into 30 mL of a solution of 2,4,6-trinitrophenol in methanol (1 in 75), previously warmed to 50°C. Allow to stand for 1 hour, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 252°C and 258°C (with decomposition).

(4)   To the aqueous layer reserved in (3) add boiling chips, and heat on a water bath for 10 minutes. Cool, add 2 mL of bromine TS, heat on a water bath for 10 minutes, and heat the solution to boil. After cooling, add 2 drops of this solution to 3 mL of a solution of resorcinol in sulfuric acid (1 in 300), and heat on a water bath for 15 minutes: a red-purple color is produced.

Purity   Heavy metals <1.07>—Proceed with 1.0 g of Prochlorperazine Maleate 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).

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 0.3 g of Prochlorperazine Maleate, previously dried, dissolve in 60 mL of acetic acid (100) while stirring and warming. Cool, and titrate <2.50> with 0.05 mol/L perchloric acid VS until the color of the solution changes from orange to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 15.15 mg of $C_{20}H_{24}ClN_3S.2C_4H_4O_4$

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

Prochlorperazine Maleate Tablets

プロクロルペラジンマレイン酸塩錠

Prochlorperazine Maleate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$: 606.09).

Method of preparation   Prepare as directed under Tablets, with Prochlorperazine Maleate.

Identification   (1)   Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 5 mg of Prochlorperazine Maleate, add 15 mL of acetic acid (100), shake, and filter. To 5 mL of the filtrate add 3 mL of sulfuric acid, and shake: a light red color develops. To this solution add 1 drop of potassium dichromate TS: a green-brown color is produced and changes to brown on standing.

(2)   Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.08 g of Prochlorperazine Maleate, add 15 mL of methanol and 1 mL of dimethylamine, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.08 g of Prochlorperazine Maleate RS in 15 mL of methanol and 1 mL of dimethylamine, 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-butanol and ammonia TS (15:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and standard solution show a red-purple color, and has the same *R*f value.

(3)   To a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.04 g of Prochlorperazine Maleate, add 10 mL of 1 mol/L hydrochloric acid TS and 20 mL of diethyl ether, shake, and centrifuge. Transfer the diethyl ether layer to a separator, wash with 5 mL of 0.05 mol/L sulfuric acid TS, and evaporate on a water bath to dryness. Dissolve the residue in 5 mL of sulfuric acid TS, filter, if necessary, and add 1 to 2 drops of potassium permanganate TS: the red color of the test solution is discharged immediately.

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

Conduct this procedure using light-resistant vessels. To 1 tablet of Prochlorperazine Maleate Tablets add $3V/5$ mL of a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1), treat with ultrasonic waves until the tablet is disintegrated, and shake vigorously for 10 minutes. Add exactly $V/20$ mL of the internal standard solution, and a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make V mL so that each mL contains about 80 μg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$). Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of prochlorperazine maleate
($C_{20}H_{24}ClN_3S.2C_4H_4O_4$)
= $M_S \times Q_T/Q_S \times V/250$

M_S: Amount (mg) of Prochlorperazine Maleate RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Prochlorperazine Maleate Tablets is not less than 75%.

Start the test with 1 tablet of Prochlorperazine 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 the dissolution medium to make exactly V' mL so that each mL contains about 9 μg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in methanol 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. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank, and determine the absorbances, A_T and A_S, at 255 nm.

Dissolution rate (%) with respect to the labeled amount of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Prochlorperazine Maleate RS taken
C: Labeled amount (mg) of prochlorperazine maleate

(C$_{20}$H$_{24}$ClN$_3$S.2C$_4$H$_4$O$_4$) in 1 tablet

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Prochlorperazine Maleate Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 8 mg of prochlorperazine maleate (C$_{20}$H$_{24}$ClN$_3$S.2C$_4$H$_4$O$_4$), add 60 mL of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1), and shake vigorously for 10 minutes. Add exactly 5 mL of the internal standard solution, and add a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Prochlorperazine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 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 prochlorperazine to that of the internal standard.

$$\text{Amount (mg) of prochlorperazine maleate}$$
$$(\text{C}_{20}\text{H}_{24}\text{ClN}_3\text{S.2C}_4\text{H}_4\text{O}_4)$$
$$= M_S \times Q_T/Q_S \times 2/5$$

M_S: Amount (mg) of Prochlorperazine Maleate RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 257 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 diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (11:9).
Flow rate: Adjust so that the retention time of prochlorperazine 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, prochlorperazine 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 prochlorperazine to that of the internal standard is not more than 1.0%.

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

Progesterone

プロゲステロン
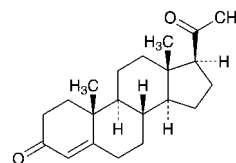
C$_{21}$H$_{30}$O$_2$: 314.46
Pregn-4-ene-3,20-dione
[*57-83-0*]

Progesterone, when dried, contains not less than 97.0% and not more than 103.0% of progesterone (C$_{21}$H$_{30}$O$_2$).

Description Progesterone occurs as white, crystals or crystalline powder.
It is soluble in methanol and in ethanol (99.5), and practically insoluble in water.
It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Progesterone 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 Progesterone 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 Progesterone, 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 Progesterone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Progesterone and Progesterone RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +184 – +194° (after drying, 0.2 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <2.60> 128 – 133°C or 120 – 122°C

Purity Related substances—Dissolve 80 mg of Progesterone in 2 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether 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 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 0.5% (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 10 mg each of Progesterone and Progesterone RS, previously dried, and dissolve each in

ethanol (99.5) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (99.5) to make exactly 50 mL, and use these solution 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 the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of progesterone } (C_{21}H_{30}O_2) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Progesterone RS taken

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

Progesterone Injection

プロゲステロン注射液

Progesterone 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 progesterone ($C_{21}H_{30}O_2$: 314.46).

Method of preparation   Prepare as directed under Injections, with Progesterone.

Description   Progesterone Injection is a clear, colorless to pale yellow, oily liquid.

Identification   To 1 mL of Progesterone Injection add 1 mL of diluted ethanol (9 in 10), shake well, take the ethanol layer, shake well with 1 mL of petroleum benzin, and use the ethanol layer as the sample solution. Separately, dissolve about 5 mg of Progesterone RS in 1 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 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 diethyl ether and diethylamine (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat the plate at 105°C for 10 minutes: the principal spot obtained from the sample solution has the same Rf value as the spot obtained from the standard solution.

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 the specific gravity of Progesterone Injection. Weigh accurately the mass of Progesterone Injection, equivalent to about 1 mL, mix with 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly V mL so that each mL contains about 0.5 mg of progesterone ($C_{21}H_{30}O_2$). Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (99.5) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Progesterone RS, previously dried in vacuum for 4 hours using phosphorus (V) oxide as the desiccant, dissolve in 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution

and ethanol (99.5) 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 progesterone to that of the internal standard.

$$\text{Amount (mg) of progesterone } (C_{21}H_{30}O_2) = M_S \times Q_T/Q_S \times V/20$$

M_S: Amount (mg) of Progesterone RS taken

Internal standard solution—A solution of testosterone propionate in ethanol (99.5) (1 in 4000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 241 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 progesterone 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, progesterone 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of progesterone to that of the internal standard is not more than 1.0%.

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

Proglumide

プログルミド
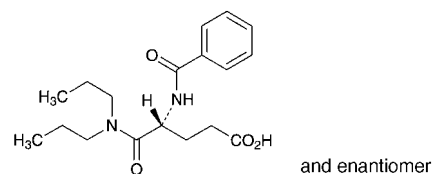
$C_{18}H_{26}N_2O_4$: 334.41
(4RS)-4-Benzoylamino-N,N-dipropylglutaramic acid
[6620-60-6]

Proglumide, when dried, contains not less than 98.5% of proglumide ($C_{18}H_{26}N_2O_4$).

Description   Proglumide occurs as white crystals or crystalline powder.

It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

A solution of Proglumide in methanol (1 in 10) shows no optical rotation.

Identification   (1)   Put 0.5 g of Proglumide in a round bottom tube, add 5 mL of hydrochloric acid, seal the tube, and heat the tube carefully at 120°C for 3 hours. After cooling,

open the tube, filter the content to collect crystals separated out, wash the crystals with 50 mL of cold water, and dry at 100°C for 1 hour: the melting point <2.60> of the crystals is between 121°C and 124°C.

(2) Determine the infrared absorption spectrum of Proglumide, 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> $E_{1\,cm}^{1\%}$ (225 nm): 384 – 414 (after drying, 4 mg, methanol, 250 mL).

Melting point <2.60>   148 – 150°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Proglumide 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>—To 1.0 g of Proglumide add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and 1.5 mL of hydrogen peroxide (30), burn the ethanol, and prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Proglumide 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, ethyl acetate, acetic acid (100) and methanol (50:18:5:4) 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 0.10% (1 g, reduced pressure, phosphorus (V) oxide, 60°C, 3 hours).

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

Assay Weigh accurately about 0.16 g of Proglumide, previously dried, dissolve in 40 mL of methanol, add 10 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
   = 33.44 mg of $C_{18}H_{26}N_2O_4$

Containers and storage Containers—Well-closed containers.

L-Proline

L-プロリン
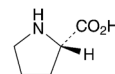
$C_5H_9NO_2$: 115.13
(2*S*)-Pyrrolidine-2-carboxylic acid
[*147-85-3*]

L-Proline contains not less than 99.0% and not more than 101.0% of L-proline ($C_5H_9NO_2$), calculated on the dried basis.

Description L-Proline occurs as white crystals or crystalline powder. It has a slightly sweet taste.

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

It is deliquescent.

Identification Determine the infrared absorption spectrum of L-Proline 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> $[\alpha]_D^{20}$: −84.0 – −86.0° (1 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54>   The pH of a solution of 1.0 g of L-Proline in 10 mL of water is 5.9 to 6.9.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Proline in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Proline. 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-Proline. 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-Proline. 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 L-Proline 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-Proline 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-Proline, 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, L-proline, 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 4 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 proline in 1 mL of the sample solution obtained from the height of the peaks obtained from the sample and standard solution: the amount of each amino acid other than proline is not more than 0.1%.

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 composed with a sulfonated polystyrene (3 μm in particle diameter) (Na type).

Column temperature: A constant temperature of about 57°C.

Chemical reaction vessel 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

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 above conditions, aspartic acid, threonine, serine, glutamic acid, proline, 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, pass 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, pass nitrogen for 5 minutes, add 81 mg of sodium borohydride, pass nitrogen for 30 minutes, and use this solution as Solution (II). Prepare a mixture with an equal volume of the Solution (I) and (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 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 deviations of the peak height of each amino acid other than proline in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time 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-Proline, 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
= 11.51 mg of $C_5H_9NO_2$

Containers and storage   Containers—Tight containers.

Promethazine Hydrochloride

プロメタジン塩酸塩

• HCl

and enantiomer
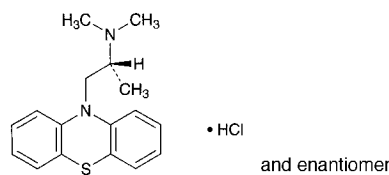
$C_{17}H_{20}N_2S \cdot HCl$: 320.88
(2*RS*)-*N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-ylamine monohydrochloride
[*58-33-3*]

Promethazine Hydrochloride, when dried, contains not less than 98.0% of promethazine hydrochloride ($C_{17}H_{20}N_2S \cdot HCl$).

Description   Promethazine Hydrochloride occurs as a white to light yellow powder.

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

It is gradually colored by light.

A solution of Promethazine Hydrochloride (1 in 25) shows no optical rotation.

Melting point: about 223°C (with decomposition).

Identification   (1)   Determine the absorption spectrum of a solution of Promethazine 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 Promethazine 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)   Dissolve 0.5 g of Promethazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. To 5 mL

of the filtrate add dilute nitric acid to make acidic: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*>   The pH of a solution of Promethazine Hydrochloride (1 in 10) is between 4.0 and 5.5.

Purity   **(1)**   Clarity and color of solution—Dissolve 1.0 g of Promethazine Hydrochloride in 10 mL of water, protecting from light: the solution is clear and colorless.

    (2)   Heavy metals <*1.07*>—Proceed with 1.0 g of Promethazine 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 under the protection from sunlight. Dissolve 0.10 g of Promethazine Hydrochloride in exactly 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 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of isopromethazine hydrochloride for thin-layer chromatography in ethanol (95) 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 the plate with a mixture of methanol and diethylamine (19:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution corresponding to the spots from the standard solution (2) are not more intense than the spot from the standard solution (2), and any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution (1).

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 Promethazine 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.
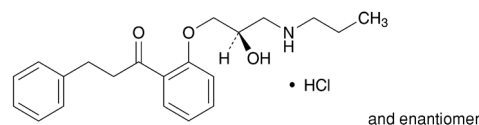
<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 32.09 mg of $C_{17}H_{20}N_2S.HCl$

</div>

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

Propafenone Hydrochloride

プロパフェノン塩酸塩

$C_{21}H_{27}NO_3.HCl$: 377.90
1-{2-[(2*RS*)-2-Hydroxy-
3-(propylamino)propyloxy]phenyl}-3-phenylpropan-1-one
monohydrochloride
[*34183-22-7*]

Propafenone Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$).

Description   Propafenone Hydrochloride occurs as white crystals or a white crystalline powder.

    It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

    A solution of Propafenone Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification   **(1)**   Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 3 mL of this solution add water to make 500 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 Propafenone 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 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 10 mL of this solution add 1 mL of dilute nitric acid, and filter to separate formed precipitate: the filtrate responds to the Qualitative Tests <*1.09*> (2) for chloride.

Melting point <*2.60*>   172 – 175°C

Purity   **(1)**   Heavy metals <*1.07*>—Proceed with 1.0 g of Propafenone 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).

    (2)   Related substances—Dissolve 0.10 g of Propafenone Hydrochloride in 20 mL of the mobile phase in the operating conditions 1, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase in the operating conditions 1 to make exactly 50 mL. Pipet 2.5 mL of this solution, add 2.5 mL of a solution of diphenyl phthalate in methanol (1 in 2000), add the mobile phase in the operating conditions 1 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 1 and 2. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of propafenone from the sample solution is not larger than the peak area of propafenone from the standard solution.

Operating conditions 1—

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 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.

Flow rate: Adjust so that the retention time of diphenyl phthalate is about 39 minutes.

Time span of measurement: Beginning after the solvent peak to the retention time of diphenyl phthalate.

System suitability 1—

System performance: Dissolve 12 mg of Propafenone Hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions 1, propafenone and isopropyl benzoate 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 1, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

Operating conditions 2—

Detector, column and column temperature: Proceed as directed in the operating conditions 1.

Mobile phase: Dissolve 7.33 g of sodium 1-decanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 700 mL of the filtrate add 700 mL of acetonitrile.

Flow rate: Adjust so that the retention time of diphenyl phthalate is about 11 minutes.

Time span of measurement: About 2.5 times as long as the retention time of diphenyl phthalate, beginning after the retention time of diphenyl phthalate.

System suitability 2—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions 2, propafenone and diphenyl phthalate are eluted in this order with the resolution between these peaks being not less than 21.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of propafenone 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 Propafenone Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 50 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
= 18.90 mg of $C_{21}H_{27}NO_3.HCl$

Containers and storage   Containers—Well-closed containers.

Propafenone Hydrochloride Tablets

プロパフェノン塩酸塩錠

Propafenone Hydrochloride Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$: 377.90).

Method of preparation   Prepare as directed under Tablets, with Propafenone Hydrochloride.

Identification   To a quantity of Propafenone Hydrochloride Tablets, equivalent to 0.3 g of Propafenone Hydrochloride, add 60 mL of water, and disintegrate by warming. After cooling, centrifuge, and to 3 mL of the supernatant liquid add water to make 500 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 302 nm and 306 nm. Separately, determine the both maximal absorbances, A_1 and A_2, of the solution, the ratio of A_1/A_2 is between 2.30 and 2.55.

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 Propafenone Hydrochloride Tablets add 30 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 6 mg of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$), add exactly 5 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 propafenone hydrochloride
($C_{21}H_{27}NO_3.HCl$)
= $M_S \times Q_T/Q_S \times 10/V$

M_S: Amount (mg) of propafenone hydrochloride for assay taken

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200).

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 Propafenone Hydrochloride Tables is not less than 75%.

Start the test with 1 tablet of Propafenone 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 0.5 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 67 μg of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 13 mg of propafenone hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 200 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 305 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 450$$

M_S: Amount (mg) of propafenone hydrochloride for assay taken

C: Labeled amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$) in 1 tablet

Assay To a quantity of Propafenone Hydrochloride Tablets, equivalent to 1.5 g of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$), add 70 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, shake well for another 5 minutes, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and centrifuge. Pipet 4 mL of the supernatant liquid, and add methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of propafenone hydrochloride for assay, previously dried at 105°C for 2 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 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 propafenone to that of the internal standard.

Amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3.HCl$)

$$= M_S \times Q_T/Q_S \times 50$$

M_S: Amount (mg) of propafenone hydrochloride for assay taken

Internal standard solution—A solution of isopropyl benzoate 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: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.

Flow rate: Adjust so that the retention time of propafenone 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, propafenone 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 propafenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Propantheline Bromide

プロパンテリン臭化物
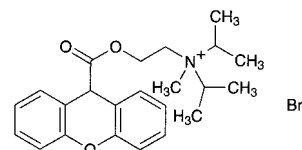
$C_{23}H_{30}BrNO_3$: 448.39
N-Methyl-*N*,*N*-bis(1-methylethyl)-2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethylaminium bromide
[*50-34-0*]

Propantheline Bromide, when dried, contains not less than 98.0% and not more than 102.0% of propantheline bromide ($C_{23}H_{30}BrNO_3$).

Description Propantheline Bromide occurs as a white to yellowish white, crystalline powder. It is odorless and has a very bitter taste.

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

The pH of a solution of 1.0 g of Propantheline Bromide in 50 mL of water is between 5.0 and 6.0.

Melting point: about 161°C (with decomposition, after drying).

Identification (1) To 5 mL of a solution of Propantheline Bromide (1 in 20) add 10 mL of sodium hydroxide TS, heat to boil for 2 minutes. Cool to 60°C, and add 5 mL of dilute hydrochloric acid. After cooling, collect the precipitates, and wash with water. Recrystallize from dilute ethanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 217°C and 222°C.

(2) Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of sulfuric acid: a vivid yellow to yellow-red color develops.

(3) To 5 mL of a solution of Propantheline Bromide (1 in 10) add 2 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (1) for bromide.

Purity Xanthene-9-carboxylic acid and xanthone—Dissolve 10 mg of Propantheline Bromide in exactly 2 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 1.0 mg of xanthene-9-carboxylic acid and 1.0 mg of xanthone in exactly 40 mL of chloroform, and use this solution as the standard solution. Perform the test immediately 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, and air-dry the plate for 10 minutes. Develop the plate with a mixture of 1,2-dichloroethane, methanol, water and formic acid (56:24:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light: the spots from the sample solution corresponding to the spots from the standard solution are not more intense than those from the standard solution.

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 4 hours).

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

Assay Weigh accurately about 1 g of Propantheline Bromide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50>
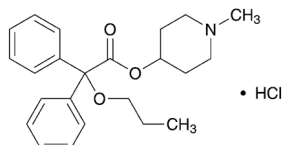
with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.84 g of $C_{23}H_{30}BrNO_3$

Containers and storage Containers—Well-closed containers.

Propiverine Hydrochloride

プロピベリン塩酸塩

$C_{23}H_{29}NO_3$.HCl: 403.94
1-Methylpiperidin-4-yl 2,2-diphenyl-2-propoxyacetate monohydrochloride
[*54556-98-8*]

Propiverine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.5% of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl).

Description Propiverine Hydrochloride occurs as white crystals or a white crystalline powder.
It is soluble in water and in ethanol (99.5).

Identification (1) Dissolve 50 mg of Propiverine Hydrochloride in 20 mL of water, and add acetonitrile 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 or the spectrum of a solution of Propiverine Hydrochroride 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 Propiverine Hydrochloride, previously dried, 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 dried Propiverine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Propiverine Hydrochloride (1 in 100) add 6 mL of ethyl acetate, and add 3 drops of silver nitrate TS: a white precipitate is formed, which does not dissolve on the addition of 0.5 mL of dilute nitric acid and shaking. The precipitate dissolves on the addition of 2 mL of ammonia TS and shaking.

Melting point <2.60>   213 – 218°C

Purity (1) Sulfate <1.14>—Perform the test with 0.40 g of Propiverine Hydrochloride. Prepare the control solution with 0.40 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 Propiverine 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 50 mg of Propiverine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample so-

lution, add the mobile phase 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. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine obtained from the standard solution, the area of the peak other than propiverine and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 1/2 times the peak area of propiverine 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 propiverine, 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 20 mL. Confirm that the peak area of propiverine obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 15 μL of the standard solution.
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 propiverine are not less than 7000 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 propiverine is not more than 2.0%.

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

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

Assay Weigh accurately about 50 mg each of Propiverine Hydrochloride and Propiverine Hydrachloride RS, both previously dried, and dissolve each in the mobile phase to make exactly 100 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 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 propiverine in each solution.

Amount (mg) of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Propiverine Hydrochloride RS taken

System suitability—
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 phenylated silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.21 g of potassium dihydrogen phosphate and 1.51 g of sodium 1-octane sulfonate in 650 mL of water, adjust to pH 3.2 with phosphoric acid, and add 350 mL of acetonitrile.

Flow rate: Adjust so that the retention time of propiverine is about 17 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 propiverine are not less than 6000 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 propiverine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Propiverine Hydrochloride Tablets

プロピベリン塩酸塩錠

Propiverine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl: 403.94).

Method of preparation Prepare as directed under Tablets, with Propiverine Hydrochloride.

Identification Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride, with 20 mL of water. Add acetonitrile to make 100 mL, centrifuge, and filter the supernatant liquid, if necessary. Determine the absorption spectrum of the supernatant liquid or the filtrate under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 257 nm and 261 nm.

Purity Related substances—Shake vigorously a quantity of powdered Propiverine Hydrochloride Tablets, equivalent to 50 mg of Propiverine Hydrochloride, with the mobile phase, add the mobile phase to make 100 mL, centrifuge, and use the supernatant liquid 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 15 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 about 0.28 to propiverine, obtained from the sample solution is not larger than 3/10 times the peak area of propiverine obtained from the standard solution, the area of the peak other than propiverine and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of propiverine from the standard solution, and the total area of the peaks other than propiverine from the sample solution is not larger than 7/10 times the peak area of propiverine 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 under Propiverine Hydrochloride.

Time span of measurement: About 2.5 times as long as the retention time of propiverine, 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 20 mL. Confirm that the peak area of propiverine obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 15 μL of the standard solution.

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 propiverine are not less than 7000 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 propiverine 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 Propiverine Hydrochloride Tablets add the mobile phase, shake vigorously, add the mobile phase to make exactly V mL so that each mL contains about 0.1 mg of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Propirevine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.

Amount (mg) of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl)
$$= M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of Propiverine Hydrochloride RS taken

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 20 minutes of Propiverine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Propiverine Hydrochloride Tablets, withdraw not less than 25 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 11 μg of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl). Pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Propiverine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, and add the dissolution medium to make exactly 100 mL. Further, pipet 15 mL of this solution, add exactly 2 mL of 0.1 mol/L hydrochloric acid TS, 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 propiverine in each solution.

Dissolution rate (%) with respect to the labeled amount of propiverine hydrochloride ($C_{23}H_{29}NO_3$.HCl)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$$

M_S: Amount (mg) of Propiverine Hydrochloride RS taken

C: Labeled amount (mg) of propiverine hydrochloride ($C_{23}H_{29}NO_3.HCl$) 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 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 diluted 0.02 mol/L potassium dihydrogen phosphate TS (1 → 2) add phosphoric acid, and adjust to pH 2.0. To 560 mL of this solution add 440 mL of acetonitrile.

Flow rate: Adjust so that the retention time of propirevine 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 propiverine 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 operations conditions, the relative standard deviation of the peak area of propiverine is not more than 2.0%.

Assay Weigh accurately and powder not less than 20 Propiverine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of propiverine hydrochloride ($C_{23}H_{29}NO_3.HCl$), add the mobile phase, shake vigorously, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Propiverine Hydrochloride RS, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Propiverine Hydrochloride.
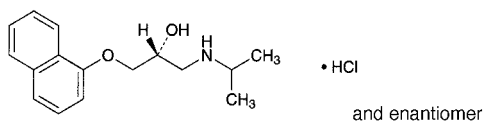
Amount (mg) of propiverine hydrochloride
($C_{23}H_{29}NO_3.HCl$)
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of Propiverine Hydrochloride RS taken

Containers and storage Containers—Tight containers.

Propranolol Hydrochloride

プロプラノロール塩酸塩

and enantiomer

$C_{16}H_{21}NO_2.HCl$: 295.80
(2*RS*)-1-(1-Methylethyl)amino-3-(naphthalen-1-yloxy)propan-2-ol monohydrochloride
[*318-98-9*]

Propranolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$).

Description Propranolol Hydrochloride occurs as a white, crystalline powder.

It is freely soluble in methanol, soluble in water and in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Propranolol Hydrochloride in methanol (1 in 40) shows no optical rotation.

It is gradualy colored to yellowish white to light brown by light.

Identification **(1)** Determine the absorption spectrum of a solution of Propranolol Hydrochloride 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.

(2) Determine the infrared absorption spectrum of Propranolol 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 Propranolol Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> The pH of a solution prepared by dissolving 0.5 g of Propranolol Hydrochloride in 50 mL of water is 5.0 – 6.0.

Melting point <*2.60*> 163 – 166°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Propranolol Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Propranolol 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 20 mg of Propranolol Hydrochloride in 10 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 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 by the automatic integration method: the area of the peak other than propranolol from the sample solution is not larger than 1/2 times the peak area of propranolol from the standard solution, and the total area of the peaks other than the peak of propranolol from the sample solution is not larger than 2 times the peak area of propranolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 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 1.6 g of sodium lauryl sulfate and 0.31 g of tetrabutylammonium dihydrogen phosphate in 450 mL of water, add 1 mL of sulfuric acid and 550 mL of acetonitrile for liquid chromatography, and adjust to pH 3.3 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust so that the retention time of propranolol is about 4 minutes.

Time span of measurement: About 5 times as long as the

retention time of propranolol.
System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propranolol obtained with 20 μL of this solution is equivalent to 17 to 33% 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 propranolol is 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 propranolol 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 Propranolol 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 in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.58 mg of $C_{16}H_{21}NO_2.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propranolol Hydrochloride Tablets

プロプラノロール塩酸塩錠

Propranolol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$: 295.80).

Method of preparation Prepare as directed under Tablets, with Propranolol Hydrochloride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 288 nm and 292 nm, and 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 tablet of Propranolol Hydrochloride Tablets add 20 mL of water, and shake until the tablet is completely disintegrated. Add 50 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and filter. 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 20 μg of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, 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 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 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of propranolol hydrochloride
($C_{16}H_{21}NO_2.HCl$)
= $M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of propranolol 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 Propranolol Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Propranolol 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 10 μg of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol 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. 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>.

Dissolution rate (%) with respect to the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of propranolol hydrochloride for assay taken
C: Labeled amount (mg) of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Propranolol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$), add 60 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Filter, discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, 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 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 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of propranolol hydrochloride
($C_{16}H_{21}NO_2.HCl$)
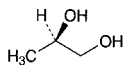= $M_S \times A_T/A_S \times 2/5$

M_S: Amount (mg) of propranolol hydrochloride for assay taken

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propylene Glycol

プロピレングリコール

and enantiomer

$C_3H_8O_2$: 76.09
(2*RS*)-Propane-1,2-diol
[*57-55-6*]

Description Propylene Glycol is a clear, colorless, viscous liquid. It is odorless, and has a slightly bitter taste.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is freely soluble in diethyl ether.

It is hygroscopic.

Identification **(1)** Mix 2 to 3 drops of Propylene Glycol with 0.7 g of triphenylchloromethane, add 1 mL of pyridine, and heat under a reflux condenser on a water bath for 1 hour. After cooling, dissolve the mixture in 20 mL of acetone by warming, shake with 0.02 g of activated charcoal, and filter. Concentrate the filtrate to about 10 mL, and cool. Collect the separated crystals, and dry in a desiccator (silica gel) for 4 hours: the crystals melt <2.60> between 174°C and 178°C.

(2) Heat gently 1 mL of Propylene Glycol with 0.5 g of potassium hydrogen sulfate: a characteristic odor is evolved.

Specific gravity <2.56> d_{20}^{20}: 1.035 – 1.040

Purity **(1)** Acidity—Mix 10.0 mL of Propylene Glycol with 50 mL of freshly boiled and cooled water, and add 5 drops of phenolphthalein TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: the solution has a red color.

(2) Chloride <1.03>—Perform the test with 2.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(3) Sulfate <1.14>—Perform the test with 10.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(4) Heavy metals <1.07>—Perform the test with 5.0 g of Propylene Glycol according to Method 1. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Propylene Glycol according to Method 1, and perform the test (not more than 2 ppm).

(6) Glycerin—Heat 1.0 g of Propylene Glycol with 0.5 g of potassium hydrogen sulfate and evaporate to dryness: no odor of acrolein is perceptible.

(7) Ethylene glycol, diethylene glycol and related substances—Weigh accurately about 5 g of Propylene Glycol, 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, and mix with methanol to make exactly 100 mL. Pipet 5 mL of this solution, and transfer to a 100-mL volumetric flask. Separately, weigh 5.0 g of propylene glycol for gas chromatography, mix with a suitable amount of methanol and put in the 100-mL volumetric flask, dilute with methanol to volume, 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 propylene glycol, 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 propylene glycol 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 Propylene Glycol taken

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 the inside surface 1 μm in thickness with 14% cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography.
  Column temperature: Inject at a constant temperature of about 100°C, rise 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 propylene glycol, beginning after the solvent peak.
System suitability—
  System performance: Mix 50 mg each of ethylene glycol, diethylene glycol and propylene glycol for gas chromatography with 100 mL of methanol. When the procedure is run with 1 μL of this mixture under the above operating conditions, ethylene glycol, propylene glycol and diethylene glycol are eluted in this order, and the resolution between the peaks of ethylene glycol and propylene glycol is not less than 5, and that between the peaks of propylene glycol and diethylene glycol is not less than 50.
  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 ethylene glycol and diethylene glycol is not more than 10%.

**Water** <2.48> Not more than 0.5% (2 g, volumetric titration, direct titration).

Residue on ignition <2.44> Weigh accurately about 20 g of Propylene Glycol in a tared crucible, and heat to boiling. Stop heating, and immediately ignite to burn. Cool, moisten the residue with 0.2 mL of sulfuric acid, and heat strongly with care to constant mass: the mass of the residue is not more than 0.005%.

Distilling range <2.57> 184 – 189°C, not less than 95 vol%.

Containers and storage Containers—Tight containers.

Propyl Parahydroxybenzoate

パラオキシ安息香酸プロピル
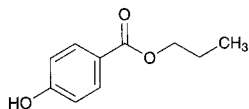
C₁₀H₁₂O₃: 180.20
Propyl 4-hydroxybenzoate
[*94-13-3*]

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 (◆ ◆).

Propyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of propyl parahydroxybenzoate ($C_{10}H_{12}O_3$).

◆**Description**   Propyl 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 Propyl 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 Propyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point *‹2.60›*   96 – 99°C

Purity   **(1)**   Clarity and color of solution—Dissolve 1.0 g of Propyl 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 Propyl 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 Propyl 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 Propyl 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 Liq-

uid 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 0.3 to propyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of propyl parahydroxybenzoate obtained from the standard solution (0.5%). For the peak area of parahydroxybenzoic acid, multiply the relative response factor, 1.4. Furthermore, the area of the peak other than propyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of propyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than propyl parahydroxybenzoate from the sample solution is not larger than 2 times the peak area of propyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of propyl 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 2.5 times as long as the retention time of propyl 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 propyl 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 propyl 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 Propyl Parahydroxybenzoate and Propyl 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 propyl parahydroxybenzoate in each solution.

Amount (mg) of propyl parahydroxybenzoate ($C_{10}H_{12}O_3$)
    $= M_S \times A_T/A_S$

M_S: Amount (mg) of Propyl 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 dihy-

drogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Propyl Parahydroxybenzoate, ethyl parahydroxybenzoate and para-hydroxybenzoic 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, ethyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid and ethyl parahydroxybenzoate to propyl parahydroxybenzoate are about 0.3 and about 0.7, respectively, and the resolution between the peaks of ethyl parahydroxybenzoate and propyl parahydroxybenzoate is not less than 3.0.

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 propyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Propylthiouracil

プロピルチオウラシル
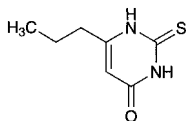
C$_7$H$_{10}$N$_2$OS: 170.23
6-Propyl-2-thiouracil
[*51-52-5*]

Propylthiouracil, when dried, contains not less than 98.0% of propylthiouracil (C$_7$H$_{10}$N$_2$OS).

Description Propylthiouracil occurs as a white powder. It is odorless, and has a bitter taste.

It is sparingly soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification (1) Shake well 0.02 g of Propylthiouracil with 7 mL of bromine TS for 1 minute, and heat until the color of bromine TS disappears. Cool, filter, and add 10 mL of barium hydroxide TS to the filtrate: a white precipitate is produced. The color of the precipitate does not turn purple within 1 minute.

(2) To 5 mL of a hot saturated solution of Propylthiouracil add 2 mL of a solution of sodium pentacyanoammine ferroate (II) *n*-hydrate (1 in 100): a green color develops.

Melting point <*2.60*> 218 – 221°C

Purity (1) Sulfate <*1.14*>—Triturate Propylthiouracil finely in a mortar. To 0.75 g of the powder add 25 mL of water, heat for 10 minutes on a water bath, cool, filter, and wash the residue with water until the volume of the filtrate becomes 30 mL. To 10 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 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.077%).

(2) Thiourea—Dissolve 0.30 g of Propylthiouracil in 50 mL of water by heating under a reflux condenser for 5 minutes, cool, and filter. To 10 mL of the filtrate add 3 mL of ammonia TS, shake well, and add 2 mL of silver nitrate TS: the solution has no more color than the following control solution.

Control solution: Weigh exactly 60 mg of thiourea, and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and proceed with 10 mL of this solution in the same manner.

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 Propylthiouracil, previously dried, and add 30 mL of water. Add 30 mL of 0.1 mol/L sodium hydroxide VS from a burette, heat to boil, and dissolve by stirring. Wash down the solid adhering to the wall of the flask with a small amount of water, and add 50 mL of 0.1 mol/L silver nitrate VS with stirring. Boil gently for 5 minutes, add 1 to 2 mL of bromothymol blue TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until a persistent blue-green color develops. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 8.512 mg of C$_7$H$_{10}$N$_2$OS

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propylthiouracil Tablets

プロピルチオウラシル錠

Propylthiouracil Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of propylthiouracil (C$_7$H$_{10}$N$_2$OS: 170.23).

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

Identification To a quantity of powdered Propylthiouracil Tablets, equivalent to 0.3 g of Propylthiouracil, add 5 mL of ammonia TS, allow to stand for 5 minutes with occasional shaking, add 10 mL of water, and centrifuge. To the supernatant liquid add acetic acid (31), collect the precipitate produced, recrystallize from water, and dry at 105°C for 1 hour: it melts <*2.60*> between 218°C and 221°C. Proceed with the residue as directed in the Identification under Propylthiouracil.

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 Propylthiouracil Tablets add 3V/4 mL of 2nd fluid for dissolution test, treat with ultrasonic waves until the tablet is disintegrated, and add 2nd fluid for dissolution test to make exactly V mL so that each mL contains about 0.25 mg of propylthiouracil (C$_7$H$_{10}$N$_2$OS). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$)
$$= M_S \times A_T/A_S \times V/200$$

M_S: Amount (mg) of propylthiouracil for assay 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 30 minutes of Propylthiouracil Tablets is not less than 80%.

Start the test with 1 tablet of Propylthiouracil 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 contains about 5.6 μg of propylthiouracil ($C_7H_{10}N_2OS$), and use this solution as the sample solution. Separately, weigh about 50 mg of propylthiouracil for assay, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 1000 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbance at 274 mm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

Dissolution rate (%) with respect to the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$)
$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$$

M_S: Amount (mg) of propylthiouracil for assay taken
C: Labeled amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$) in 1 tablet

Assay   Weigh accurately the mass of not less than 20 Propylthiouracil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of propylthiouracil ($C_7H_{10}N_2OS$), add 150 mL of 2nd fluid for dissolution test, disperse finely the particles with the aid of ultrasonic waves, and add 2nd fluid for dissolution test to make exactly 200 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, pipet 2 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propylthiouracil for assay, previously dried at 105°C for 2 hours, and dissolve in 2nd fluid for dissolution test to make exactly 200 mL. Pipet 2 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbance at 274 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

Amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$)
$$= M_S \times A_T/A_S$$

M_S: Amount (mg) of propylthiouracil for assay taken

Containers and storage   Containers—Well-closed containers.
Storage—Light-resistant.

Protamine Sulfate

プロタミン硫酸塩

Protamine Sulfate is the sulfate of protamine prepared from the mature spermary of fish belonging to the family *Salmonidae*.
It has a property to bind with heparin.
It binds with not less than 100 Units of heparin per mg, calculated on the dried basis.

Description   Protamine Sulfate occurs as a white powder.
It is sparingly soluble in water.

Identification   (1)   Dissolve 1 mg of Protamine Sulfate in 2 mL of water, add 5 drops of a solution prepared by dissolving 0.1 g of 1-naphthol in 100 mL of diluted ethanol (7 in 10) and 5 drops of sodium hypochlorite TS, then add sodium hydroxide TS until the solution becomes alkaline: a vivid red color develops.
(2)   Dissolve 5 mg of Protamine Sulfate in 1 mL of water by warming, add 1 drop of a solution of sodium hydroxide (1 in 10) and 2 drops of copper (II) sulfate TS: a red-purple color develops.
(3)   An aqueous solution of Protamine Sulfate (1 in 20) responds to the Qualitative Tests *<1.09>* for sulfate.

pH *<2.54>*   Dissolve 1.0 g of Protamine Sulfate in 100 mL of water: the pH of this solution is between 6.5 and 7.5.

Purity   (1)   Clarity and color of solution—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water: the solution is clear and colorless.
(2)   Absorbance—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: the absorbance between 260 nm and 280 nm is not more than 0.1.

Loss on drying *<2.41>*   Not more than 5.0% (1 g, 105°C, 3 hours).

Nitrogen content   Weigh accurately about 10 mg of Protamine Sulfate, and perform the test as directed under Nitrogen Determination *<1.08>*: the amount of nitrogen (N:14.01) is 22.5 – 25.5%, calculated on the dried basis.

Heparin-binding capacity
(i)   Sample solution (a)—Weigh accurately about 15 mg of Protamine Sulfate, and dissolve in water to make exactly 100 mL. Repeat this procedure 3 times, and use the solutions so obtained as the sample solutions (a_1), (a_2) and (a_3).
(ii)   Sample solution (b)—Pipet 10 mL each of the sample solutions (a_1), (a_2) and (a_3), add exactly 5 mL of water to them, and use these solutions as the sample solutions (b_1), (b_2) and (b_3).
(iii)   Sample solution (c)—Pipet 10 mL each of the sample solutions (a_1), (a_2) and (a_3), add exactly 20 mL of water to them, and use these solutions as the sample solutions (c_1), (c_2) and (c_3).
(iv)   Standard solution—Dissolve Heparin Sodium RS in water to make a solution containing exactly about 20 Units per mL.
(v)   Procedure—Transfer exactly 2 mL of the sample solution to a cell for spectrophotometer, add the standard solution dropwise while mixing, and determine the transmittance at 500 nm as directed under Ultraviolet-visible Spectrophotometry *<2.24>*. Continue the addition until a sharp change in the transmittance is observed, and note the

volume, *V* mL, of the standard solution added. Repeat this procedure 2 times for each sample solution.

(vi) Calculation—Calculate the amount of heparin bound with 1 mg of the sample by the following formula from the volume of titrant on each sample solution, and calculate the average of 18 results obtained. The assay is not valid unless each relative standard deviation of 6 results obtained from the sample solution (a), sample solution (b) and sample solution (c) is not more than 5%, respectively, and also unless each relative standard deviation of 6 results obtained from 3 sets, (a_1, b_1, c_1), (a_2, b_2, c_2) and (a_3, b_3, c_3) is not more than 5%, respectively.

Amount (heparin Unit) of heparin bound to 1 mg
of Protamine Sulfate
$$= S \times V \times 50/M_T \times d$$

S: Amount (heparin Unit) of heparin sodium in 1 mL of the standard solution

M_T: Amount (mg) of Protamine Sulfate taken, calculated on the dried basis

d: Dilution factor for each sample solution from the sample solution (a)

Sulfate content   Weigh accurately about 0.15 g of Protamine Sulfate, dissolve in 75 mL of water, add 5 mL of 3 mol/L hydrochloric acid TS, and heat to boil. Add gradually 10 mL of barium chloride TS while boiling, and allow to stand for 1 hour while heating. Filter the precipitate formed, wash the precipitate with warm water several times, and transfer the precipitate into a tared crucible. Dry the precipitate, and incinerate by ignition to constant mass: the amount of sulfate (SO_4) is 16 – 22%, calculated on the dried basis, where 1 g of the residue is equivalent to 0.4117 g of SO_4.

Containers and storage   Containers—Tight containers.

Protamine Sulfate Injection

プロタミン硫酸塩注射液

Protamine Sulfate Injection is an aqueous injection.
It contains not less than 92.0% and not more than 108.0% of the labeled amount of Protamine Sulfate. It binds with not less than 100 Units of heparin per mg of the labeled amount.

Method of preparation   Prepare as directed under Injections, with Protamine Sulfate.

Description   Protamine Sulfate Injection is a colorless liquid. It is odorless or has the odor of preservatives.

Identification   (1)   Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of Protamine Sulfate, with water to make 2 mL, and proceed as directed in the Identification (1) under Protamine Sulfate.

(2)   Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

pH *<2.54>*   5.0 – 7.0

Bacterial endotoxins *<4.01>*   Less than 6.0 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   (1)   Protein—Pipet a volume of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate, transfer to a Kjeldahl flask, evaporate on a water bath to dryness with the aid of a current of air, determine the nitrogen as directed under Nitrogen Determination *<1.08>*, and calculate the amount of protein by converting 0.24 mg of nitrogen (N: 14.01) to 1 mg of protein.

(2)   Heparin-binding activity—Proceed the test as directed in the Heparin-binding capacity under Protamine Sulfate, changing the sample solution (a) as below, and determine the amount of heparin bound to 1 mg of protein by dividing by the amount of protein.

(i) Sample solution (a)—Pipet a volume of Protamine Sulfate Injection, equivalent to 15.0 mg of Protamine Sulfate, and add water to make exactly 100 mL. Repeat this procedure two more times, and designate the solutions so obtained as the sample solutions (a_1), (a_2) and (a_3).

Containers and storage   Containers—Hermetic containers.

Prothionamide

プロチオナミド
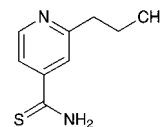
$C_9H_{12}N_2S$: 180.27
2-Propylpyridine-4-carbothioamide
[*14222-60-7*]

Prothionamide, when dried, contains not less than 98.0% of prothionamide ($C_9H_{12}N_2S$).

Description   Prothionamide occurs as yellow crystals or crystalline powder. It has a slight, characteristic odor.
It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.
It dissolves in dilute hydrochloric acid and in dilute sulfuric acid.

Identification   (1)   Mix 0.05 g of Prothionamide with 0.1 g of 1-chloro-2,4-dinitrobenzene, transfer about 10 mg of this mixture to a test tube, and heat for several seconds over a small flame until the mixture is fused. Cool, and add 3 mL of potassium hydroxide-ethanol TS: a red to orange-red color develops.

(2)   Place 0.5 g of Prothionamide in a 100-mL beaker, and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently, and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100), and heat on a water bath: the gas evolved darkens moistened lead (II) acetate paper. Evaporate the solution on a water bath to 3 to 5 mL with the aid of a current of air, cool, add 10 mL of water, and mix well. Filter the crystals by suction, recrystallize from water immediately, and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt *<2.60>* between 198°C and

203°C (with decomposition).

Melting point <2.60>   142 – 145°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Prothionamide in 20 mL of ethanol (95): the solution is clear, and shows a yellow color.

(2) Acidity—Dissolve 3.0 g of Prothionamide in 20 mL of methanol with warming. Add 100 mL of water to the solution, cool in an ice water bath with agitation, and remove any precipitate by filtration. Allow 80 mL of the filtrate to cool to room temperature, and add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Prothionamide 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 0.6 g of Prothionamide according to Method 3, and perform the test. To the test solution 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 to burn (not more than 3.3 ppm).

Loss on drying <2.41>   Not more than 0.5% (1 g, 80°C, 3 hours).

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

Assay Weigh accurately about 0.3 g of Prothionamide, 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.
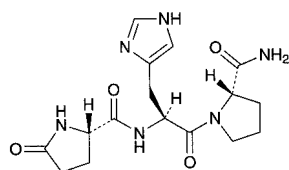
Each mL of 0.1 mol/L perchloric acid VS
= 18.03 mg of $C_9H_{12}N_2S$

Containers and storage   Containers—Well-closed containers.

Storage—Light-resistant.

Protirelin

プロチレリン

$C_{16}H_{22}N_6O_4$: 362.38
5-Oxo-L-prolyl-L-histidyl-L-prolinamide
[24305-27-9]

Protirelin contains not less than 98.5% of protirelin ($C_{16}H_{22}N_6O_4$), calculated on the anhydrous basis.

Description   Protirelin occurs as a white powder.

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

It is hygroscopic.

Identification **(1)** Take 0.01 g of Protirelin in a test tube made of hard glass, add 0.5 mL of 6 mol/L hydrochloric acid TS, seal the upper part of the tube, and heat carefully at 110°C for 5 hours. After cooling, open the seal, transfer the contents into a beaker, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 0.08 g of L-glutamic acid, 0.12 g of L-histidine hydrochloride monohydrate and 0.06 g of L-proline in 20 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 a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the three spots obtained from the sample solution show the same color and the same *R*f value as each corresponding spots obtained from the standard solution.

(2) Determine the infrared absorption spectrum of Protirelin, 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> $[\alpha]_D^{20}$: $-66.0 - -69.0°$ (0.1 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54>   Dissolve 0.20 g of Protirelin in 10 mL of water: the pH of this solution is between 7.5 and 8.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Protirelin in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Protirelin 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 Protirelin 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 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 (1) of silica gel for thin-layer chromatography, and spot 5 μL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of 1-butanol, water, pyridine and acetic acid (100) (4:2:1:1) to a distance of about 12 cm, and dry the plates at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plates. Successively spray evenly a solution of sodium carbonate decahydrate (1 in 10) on it: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and heat at 80°C for 5 minutes: no colored spot appears.

Water <2.48>   Not more than 5.0% (0.1 g, volumetric titration, direct titration).

**Residue on ignition** <2.44>   Not more than 0.3% (0.2 g).

Assay Weigh accurately about 70 mg of Protirelin 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
  = 7.248 mg of $C_{16}H_{22}N_6O_4$

Containers and storage   Containers—Tight containers.

Protirelin Tartrate Hydrate

プロチレリン酒石酸塩水和物
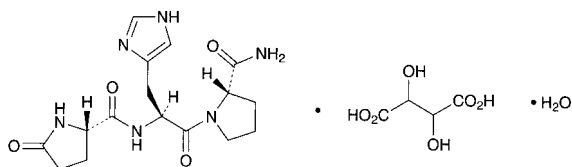
$C_{16}H_{22}N_6O_4.C_4H_6O_6.H_2O$: 530.49
5-Oxo-L-prolyl-L-histidyl-L-prolinamide monotartrate
monohydrate
[*24305-27-9*, Protirelin]

Protirelin Tartrate Hydrate, calculated on the anhydrous basis, contains not less than 98.5% of protirelin tartrate ($C_{16}H_{22}N_6O_4.C_4H_6O_6$: 512.48).

Description   Protirelin Tartrate Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

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

Melting point: about 187°C (with decomposition).

Identification   (1)   To 1 mL of a solution of Protirelin Tartrate Hydrate (1 in 1000) add 2 mL of a solution of 4-nitrobenzene diazonium fluoroborate (1 in 2000) and 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.0): a red color develops.

(2)   Dissolve 0.03 g of Protirelin Tartrate Hydrate in 5 mL of sodium hydroxide TS, add 1 drop of copper (II) sulfate TS: a purple color develops.

(3)   To 0.20 g of Protirelin Tartrate Hydrate add 5.0 mL of 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the sample solution. Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride monohydrate and 17 mg of L-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating, 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 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry at 80°C for 5 minutes: the three spots obtained from the sample solution show, respectively, the same color and the same Rf value as the corresponding spot obtained from the standard solution.

(4)   A solution of Protirelin Tartrate Hydrate (1 in 40) responds to the Qualitative Tests <1.09> for tartrate.

Optical rotation <2.49> $[\alpha]_D^{20}$: −50.0 – −53.0° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54>   Dissolve 1.0 g of Protirelin Tartrate 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 0.10 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless.

(2)   Heavy metals <1.07>—Proceed with 1.0 g of Protirelin Tartrate 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>—Take 1.0 g of Protirelin Tartrate Hydrate in a porcelain crucible. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol, and heat gradually to incinerate. If a carbonized material still remains in this method, moisten with a small quantity of nitric acid, and ignite to incinerate. After cooling, add 10 mL of dilute hydrochloric acid, heat on a water bath to dissolve the residue, use this solution as the test solution, and perform the test (not more than 2 ppm).

(4)   Related substances—Dissolve 0.60 g of Protirelin Tartrate 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 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 (1) of silica gel for thin-layer chromatography. Spot 5 µL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of chloroform, methanol and ammonia solution (28) (6:4:1) to a distance of about 10 cm, and dry at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plate. Then, spray evenly a solution of sodium carbonate decahydrate (1 in 10) on the plate: the spots other than the principal spot from the sample solution are not more intense than those from the standard solution in color. On the other hand, spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and dry at 80°C for 5 minutes: no colored spot is obtained.

Water <2.48>   Not more than 4.5% (0.2 g, volumetric titration, direct titration).

**Residue on ignition** <2.44>   Not more than 0.2% (0.5 g).

Assay   Weigh accurately about 0.5 g of Protirelin Tartrate Hydrate, dissolve in 80 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
  = 51.25 mg of $C_{16}H_{22}N_6O_4.C_4H_6O_6$

Containers and storage   Containers—Well-closed containers.

Pullulan

プルラン
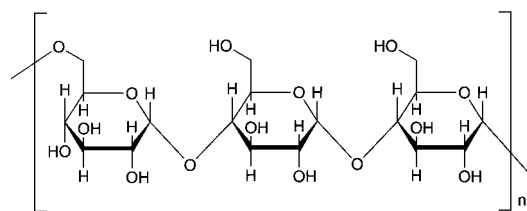
(C₁₈H₃₀O₁₅)_n

$(C_{18}H_{30}O_{15})_n$

Poly[6)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→]
[*9057-02-7*]

Pullulan is a neutral simple polysaccharide produced by the growth of *Aureobasidium pullulans*. It has a chain structure of repeated α-1,6 binding of maltotriose composed of three glucoses in α-1,4 binding.

Description Pullulan occurs as a white powder.

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

Identification (1) Dissolve 10 g of Pullulan in 100 mL of water with stirring by adding in small portions: a viscous solution is produced.

(2) Mix 10 mL of the viscous solution obtained in (1) with 0.1 mL of pullulanase TS, and allow to stand: the solution loses its viscosity.

(3) To 10 mL of a solution of Pullulan (1 in 50) add 2 mL of macrogol 600: a white precipitate is formed immediately.

Viscosity <*2.53*> Take exactly 10.0 g of Pullulan, previously dried, dissolve in water to make exactly 100 g, and perform the test at 30 ± 0.1°C as directed in Method 1: the kinematic viscosity is between 100 mm^2/s and 180 mm^2/s.

pH <*2.54*> Dissolve 1.0 g of Pullulan in 10 mL of freshly boiled and cooled water: the pH is between 4.5 and 6.5.

Purity (1) Heavy metals <*1.07*>—Proceed with 4.0 g of Pullulan 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).

(2) Nitrogen—Weigh accurately about 3 g of Pullulan, previously dried, and perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is not more than 0.05%. Use 12 mL of sulfuric acid for the decomposition, and add 40 mL of a solution of sodium hydroxide (2 in 5).

(3) Monosaccharide and oligosaccharides—Dissolve 0.8 g of Pullulan, previously dried, in 100 mL of water, and designate this solution as the sample stock solution. To 1 mL of the sample stock solution add 0.1 mL of potassium chloride saturated solution, and shake vigorously with 3 mL of methanol. Centrifuge, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of the sample stock solution, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 0.2 mL each of the sample solution, the standard solution and water, transfer them gently to each test tube containing 5 mL of a solution of anthrone in diluted sulfuric acid (3 in 4) (1 in 500) and cooling in ice water, stir immediately, then heat at 90°C for 10 minutes, and cool immediately. Perform the test with

these solutions so obtained as directed under Ultraviolet-visible Spectrophtometry <*2.24*> using water as a blank, and determine the absorbances at 620 nm, A_T, A_S and A_B: the amount of monosaccharide and oligosaccharides is not more than 10.0%.

Amount (%) of monosaccharide and oligosaccharides
$$= (A_T - A_B)/(A_S - A_B) \times 8.2$$
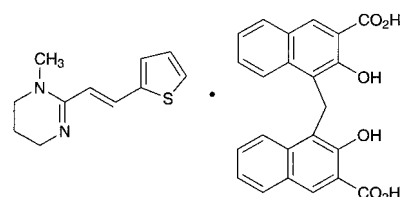
Loss on drying <*2.41*> Not more than 6.0% (1 g, in vacuum, 90°C, 6 hours).

Residue on ignition <*2.44*> Not more than 0.3% (2 g).

Containers and storage Containers—Well-closed containers.

Pyrantel Pamoate

ピランテルパモ酸塩

C₁₁H₁₄N₂S.C₂₃H₁₆O₆: 594.68

$C_{11}H_{14}N_2S.C_{23}H_{16}O_6$: 594.68

1-Methyl-2-[(1*E*)-2-(thien-2-yl)vinyl]-1,4,5,6-tetrahydropyrimidine mono[4,4′-methylenebis(3-hydroxy-2-naphthoate)]
[*22204-24-6*]

Pyrantel Pamoate, when dried, contains not less than 98.0% of pyrantel pamoate (C₁₁H₁₄N₂S. C₂₃H₁₆O₆).

Description Pyrantel Pamoate occurs as a light yellow to yellow, crystalline powder. It is odorless and tasteless.

It is sparingly soluble in *N*,*N*-dimethylformamide, very slightly soluble in methanol and in ethanol (95), and practically insoluble in water, in ethyl acetate and in diethyl ether.

Melting point: 256 – 264°C (with decomposition).

Identification (1) To 0.05 g of Pyrantel Pamoate add 10 mL of methanol and 1 mL of a mixture of hydrochloric acid and methanol (1:1), and shake vigorously: a yellow precipitate is produced. Filter the solution, and use the filtrate as the sample solution. Use the precipitate for the test (2). To 0.5 mL of the sample solution add 1 mL of a solution of 2,3-indolinedione in sulfuric acid (1 in 1000): a red color develops.

(2) Collect the precipitate obtained in the test (1), wash with methanol, and dry at 105°C for 1 hour. To 0.01 g of the dried precipitate add 10 mL of methanol, shake well, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color develops.

(3) Dissolve 0.1 g of Pyrantel Pamoate in 50 mL of *N*,*N*-dimethylformamide, and add methanol to make 200 mL. To 2 mL of the solution add a solution of hydrochloric acid in methanol (9 in 1000) 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.

(4) Determine the infrared absorption spectrum of Pyrantel Pamoate, previously dried, as directed in the potas-

sium 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>—To 1.0 g of Pyrantel Pamoate add 10 mL of dilute nitric acid and 40 mL of water, and heat on a water bath with shaking for 5 minutes. After cooling, add water to make 50 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Proceed 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)   Sulfate <1.14>—To 0.75 g of Pyrantel Pamoate add 5 mL of dilute hydrochloric acid and water to make 100 mL, and heat on a water bath for 5 minutes with shaking. After cooling, add water to make 100 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.144%).

   (3)   Heavy metals <1.07>—Proceed with 1.0 g of Pyrantel Pamoate 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).

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

   (5)   Related substances—The procedure should be performed under protection from light in light-resistant vessels. Dissolve 0.10 g of Pyrantel Pamoate in 10 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 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, 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 spot of pyrantel and the spot of pamoic acid from the sample solution are not more intense than the spot of pyrantel (Rf value: about 0.3) from the standard solution.

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

Residue on ignition <2.44>   Not more than 0.3% (1 g).

Assay   Weigh accurately about 0.5 g of Pyrantel Pamoate, previously dried, add 25 mL of chloroform and 25 mL of sodium hydroxide TS, shake for 15 minutes, and extract. Extract further with two 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, add 30 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.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 59.47 mg of $C_{11}H_{14}N_2S.C_{23}H_{16}O_6$

</div>

Containers and storage   Containers—Tight containers.

Pyrazinamide

ピラジナミド
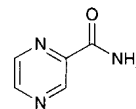
$C_5H_5N_3O$: 123.11
Pyrazine-2-carboxamide
[98-96-4]

   Pyrazinamide, when dried, contains not less than 99.0% and not more than 101.0% of pyrazinamide ($C_5H_5N_3O$).

Description   Pyrazinamide occurs as white crystals or crystalline powder.

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

Identification   (1)   Determine the absorption spectrum of a solution of Pyrazinamide 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 Pyrazinamide, 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>   188 – 193°C

Purity   (1)   Heavy metals <1.07>—Proceed with 1.0 g of Pyrazinamide 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 Pyrazinamide 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 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 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 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 0.5% (1 g, in vacuum, silica gel, 4 hours).

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

Assay   Weigh accurately about 0.1 g of Pyrazinamide, 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.
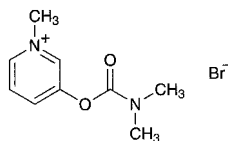
<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 12.31 mg of $C_5H_5N_3O$

</div>

Containers and storage   Containers—Well-closed contain-

ers.

Pyridostigmine Bromide

ピリドスチグミン臭化物

C₉H₁₃BrN₂O₂: 261.12
3-Dimethylcarbamoyloxy-1-methylpyridinium bromide
[*101-26-8*]

Pyridostigmine Bromide, when dried, contains not less than 98.5% of pyridostigmine bromide ($C_9H_{13}BrN_2O_2$).

Description Pyridostigmine Bromide occurs as a white, crystalline powder. It is odorless or has a slightly characteristic odor.

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

The pH of a solution of 1.0 g of Pyridostigmine Bromide in 10 mL of water is between 4.0 and 6.0.

It is deliquescent.

Identification (1) Dissolve 0.02 g of Pyridostigmine Bromide in 10 mL of water, add 5 mL of Reinecke salt TS: a light red precipitate is produced.

(2) To 0.1 g of Pyridostigmine Bromide add 0.6 mL of sodium hydroxide TS: the unpleasant odor of dimethylamine is perceptible.

(3) Determine the absorption spectrum of a solution of Pyridostigmine Bromide in 0.1 mol/L hydrochloric acid TS (1 in 30,000) 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) A solution of Pyridostigmine Bromide (1 in 50) responds to the Qualitative Tests <*1.09*> for Bromide.

Melting point <*2.60*> 153 – 157°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pyridostigmine Bromide in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Pyridostigmine Bromide 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 Pyridostigmine Bromide according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pyridostigmine Bromide in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) 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 methanol, chloroform and ammonium

chloride TS (5:4:1) 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 in color.

Loss on drying <*2.41*> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 100°C, 5 hours).

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

Assay Weigh accurately about 0.3 g of Pyridostigmine Bromide, 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.
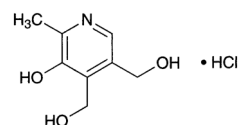
Each mL of 0.1 mol/L perchloric acid VS
= 26.11 mg of $C_9H_{13}BrN_2O_2$

Containers and storage Containers—Hermetic containers.

Pyridoxine Hydrochloride

Vitamin B₆

ピリドキシン塩酸塩

C₈H₁₁NO₃.HCl: 205.64
4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol
monohydrochloride
[*58-56-0*]

Pyridoxine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl).

Description Pyridoxine Hydrochloride occurs as a white to pale yellow, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

It is gradually affected by light.

Melting point: about 206°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Pyridoxine 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 or the spectrum of a solution of Pyridoxine 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 Pyridoxine Hydrochloride, previously dried, 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 Pyridoxine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pyridoxine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> The pH of a solution prepared by dissolving

1.0 g of Pyridoxine Hydrochloride in 50 mL of water is between 2.5 and 3.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pyridoxine hydrochloride in 20 mL of water: the solution is clear and colorless.

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

(3) Related substances—Dissolve 1.0 g of Pyridoxine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add 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 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate, and air-dry: 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 0.30% (1 g, in vacuum, silica gel, 4 hours).

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

Assay Weigh accurately about 0.2 g of Pyridoxine Hydrochloride, previously dried, add 5 mL of acetic acid (100) and 5 mL of acetic anhydride, dissolve by gentle boiling, cool, add 30 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.

<div align="center">

Each mL of 0.1 mol/L perchloric acid VS
= 20.56 mg of C$_8$H$_{11}$NO$_3$.HCl

</div>

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

Pyridoxine Hydrochloride Injection

Vitamin B$_6$ Injection

ピリドキシン塩酸塩注射液

Pyridoxine Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pyridoxine hydrochloride (C$_8$H$_{11}$NO$_3$.HCl: 205.64).

Method of preparation   Prepare as directed under Injections, with Pyridoxine Hydrochloride.

Description   Pyridoxine Hydrochloride Injection is a colorless or pale yellow, clear liquid.
It is gradually affected by light.
pH: 3.0 – 6.0

Identification   (1)   To a volume of Pyridoxine Hydrochlo-

ride Injection, equivalent to 0.05 g of Pyridoxine Hydrochloride, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.1 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 288 nm and 292 nm.

(2) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.01 g of Pyridoxine Hydrochloride, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.01 g of Pyridoxine Hydrochloride RS 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 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate: the spots obtained from the sample solution and the standard solution are blue in color and have the same Rf value.

Bacterial endotoxins <4.01>   Less than 3.0 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   Measure exactly a volume of Pyridoxine Hydrochloride Injection, equivalent to about 20 mg of pyridoxine hydrochloride (C$_8$H$_{11}$NO$_3$.HCl), dilute with water, if necessary, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Pyridoxine Hydrochloride RS, previously dried in a desiccator (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 200 mL, and use this solution as the standard solution. Pipet 1 mL each of the sample solution and standard solution, add 2.0 mL of barbital buffer solution, 9.0 mL of 2-propanol and 2.0 mL of a freshly prepared solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000), shake well, add 2-propanol to make exactly 25 mL, and allow to stand for 90 minutes. Determine the absorbances, A_T and A_S, of the subsequent sample solution and subsequent standard solution, respectively, at 650 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner with 1 mL of water, as the blank.

<div align="center">

Amount (mg) of pyridoxine hydrochloride
(C$_8$N$_{11}$NO$_3$.HCl)
= $M_S \times A_T/A_S \times 1/5$

</div>

M_S: Amount (mg) of Pyridoxine Hydrochloride RS taken

Containers and storage   Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Pyroxylin

ピロキシリン

Pyroxylin is a nitric acid ester of cellulose. It is usually moistened with 2-propanol or some other solvent.

Description   Pyroxylin occurs as a white cotton-like substance or white flakes.

It is freely soluble in acetone, and very slightly soluble in diethyl ether.

Upon heating or exposure to light, it is decomposed with the evolution of nitrous acid vapors.

Identification   Ignite Pyroxylin: it burns very rapidly with a luminous flame.

Purity   **(1)**   Clarity of solution—Dissolve 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, in 25 mL of a mixture of diethyl ether and ethanol (95) (3:1): the solution is clear.

(2)   Acidity—Shake 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, with 20 mL of water for 10 minutes: the filtrate is neutral.

(3)   Water-soluble substances—Evaporate 10 mL of the filtrate obtained in (2) on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.5 mg.

(4)   Residue on ignition—Weigh accurately about 2 g of Pyroxylin, previously dried at 80°C for 2 hours, and moisten with 10 mL of a solution of castor oil in acetone (1 in 20) to gelatinize the sample. Ignite the contents to carbonize the sample, heat strongly at about 500°C for 2 hours, and allow to cool in a desicator (silica gel): the amount of the residue is not more than 0.30%.

Containers and storage   Containers—Tight containers.

Storage—Light-resistant, packed loosely, remote from fire, and preferably in a cold place.

Pyrrolnitrin

ピロールニトリン
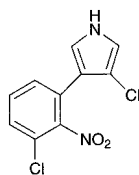
C$_{10}$H$_6$Cl$_2$N$_2$O$_2$: 257.07
3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole
[*1018-71-9*]

Pyrrolnitrin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin (C$_{10}$H$_6$Cl$_2$N$_2$O$_2$).

Description   Pyrrolnitrin occurs as yellow to yellow-brown, crystals or 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 Pyrrolnitrin 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 Pyrrolnitrin 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 Pyrrolnitrin 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 Pyrrolnitrin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60>   124 – 128°C

Purity   Related substances—Dissolve 0.10 g of Pyrrolnitrin 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 100 mL. Pipet 3 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 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 xylene, ethyl acetate and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 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 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 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 an amount of Pyrrolnitrin and Pyrrolnitrin RS, equivalent to about 50 mg (potency) each, and dissolve separetely in diluted acetonitrile (3 in 5) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add diluted acetonitrile (3 in 5) 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 pyrrolnitrin to that of the internal standard.

Amount [μg (potency)] of pyrrolnitrin (C$_{10}$H$_6$Cl$_2$N$_2$O$_2$)
    $= M_S \times Q_T/Q_S \times 1000$

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

Internal standard solution—A solution of benzyl benzoate in diluted acetonitrile (3 in 5) (3 in 500).
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 25°C.
Mobile phase: A mixture of water and acetonitrile (11:9).
Flow rate: Adjust so that the retention time of pyrrolnitrin 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, pyrrolnitrin 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pyrrolnitrin to that of the internal standard is not more than 1.0%.

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

Quetiapine Fumarate

クエチアピンフマル酸塩
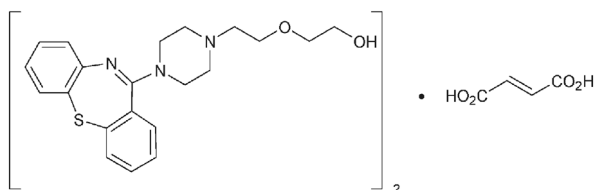
($C_{21}H_{25}N_3O_2S)_2.C_4H_4O_4$: 883.09
2-[2-(4-Dibenzo[*b*,*f*][1,4]thiazepin-11-ylpiperazin-1-yl)ethoxy]ethanol hemifumarate
[*111974-72-2*]

Quetiapine Fumarate contains not less than 98.0% and not more than 102.0% of quetiapine fumarate [($C_{21}H_{25}N_3O_2S)_2.C_4H_4O_4$], calculated on the anhydrous basis.

Description   Quetiapine Fumarate occurs as a white powder.

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

Identification   (1)   Determine the absorption spectrum of a solution of Quetiapine Fumarate in a mixture of water and acetonitrile (1:1) (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 Quetiapine Fumarate 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 Quetiapine Fumarate 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 Quetiapine Fumarate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3)   Dissolve 40 mg of Quetiapine Fumarate and 10 mg of fumaric acid for thin-layer chromatography in separate 10 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 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 spot having a larger *R*f value among the spots obtained with the sample solution and the spot obtained with the standard solution show the same *R*f value.

Purity   (1)   Heavy metals <1.07>—Proceed with 2.0 g of

Quetiapine Fumarate 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   (i)—To 20 mg of Quetiapine Fumarate add 30 mL of the mobile phase, dissolve with the aid of ultrasonic waves, add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, and add the mobile phase 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. Determine each peak area by the automatic integration method, and calculate the amount of each related substance by the following equation: the amount is not more than 0.10%. For the area of the peaks, having a relative retention time of about 0.5 and about 0.9 to quetiapine, multiply their relative response factors, 0.6 and 0.9, respectively.

Amount (%) of each related substance $= A_T/A_S \times 1/2$

A_S: Peak area of quetiapine obtained with the standard solution
A_T: Each peak area other than quetiapine obtained with the sample 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 1.8 times as long as the retention time of quetiapine, 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 50 mL. Confirm that the peak area of quetiapine obtained with 50 μL of this solution is equivalent to 7 to 13% of that obtained with 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 quetiapine are not less than 6000 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 quetiapine is not more than 2.0%.

(ii)—To 20 mg of Quetiapine Fumarate add 30 mL of a mixture of acetonitrile, water and the mobile phase (2:1:1), dissolve with the aid of ultrasonic waves, add the same mixture to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the same mixture to make exactly 100 mL. Pipet 5 mL of this solution, add the same mixture 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. Determine each peak area by the automatic integration method, and calculate the amount of each related substance by the following equation: the amount is not more than 0.10%. For the area of the peak, having a relative retention time of about 1.9 to quetiapine, multiply its relative response factor, 0.8.

Amount (%) of each related substance $= A_T/A_S \times 1/2$