(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

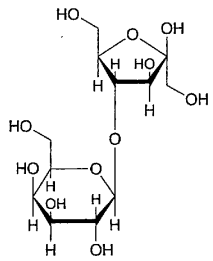
(3) Standard solutions—Weigh accurately an amount of Kitasamycin Reference Standard equivalent to about 0.03 g (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Kitasamycin equivalent to about 0.03 g (potency), dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of the solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Lactulose

ラクツロース



$C_{12}H_{22}O_{11}$: 342.30

4-*O*-β-D-Galactopyranosyl-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 $C_{12}H_{22}O_{11}$.

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 1 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 To 2.0 g of Lactulose add water to make 15 mL: the pH of the solution is between 3.5 and 5.5.

Specific gravity d_{20}^{20}: 1.320 - 1.360

Purity **(1)** Heavy metals—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—Prepare the test solution with 1.0 g of Lactulose according to Method 1, and perform the test using Apparatus B (not more than 2 ppm).

(3) Other succharides—Determine the heights of the peaks corresponding to D-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 D-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 D-galactose of not more than 11%, and lactose of not more than 6%.

Amount (mg) of D-galactose ($C_6H_{12}O_6$)
= amount (mg) of D-galactose
$\times \frac{Q_{Ta}}{Q_{Sa}}$

Amount (mg) of lactose ($C_{12}H_{22}O_{11}.H_2O$)
= amount (mg) of lactose monohydrate
$\times \frac{Q_{Tb}}{Q_{Sb}}$

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

Residue on ignition Not more than 0.10% (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 Reference Standard, accurately about 0.08 g of D-galactose and accurately about 0.04 g 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 the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of lactulose to that of the internal standard, respectively.

Amount (mg) of $C_{12}H_{22}O_{11}$
= amount (mg) of Lactulose Reference Standard
$\times \frac{Q_T}{Q_S}$

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 strong acid 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 the flow rate 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.

Lanatoside C

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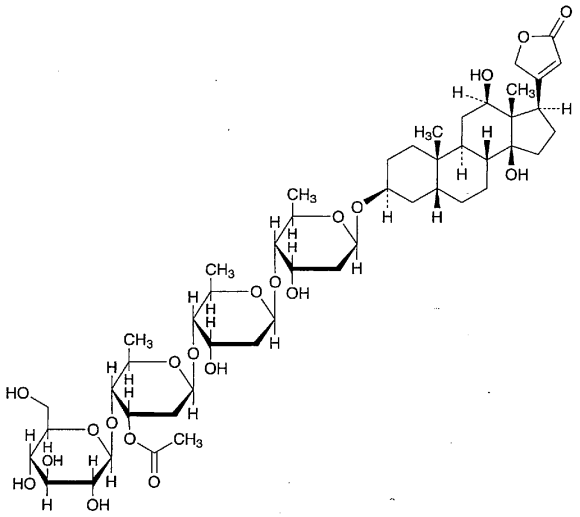

$C_{49}H_{76}O_{20}$: 985.12

3β-[*O*-β-D-Glucopyranosyl-(1→4)-*O*-3-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-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 $C_{49}H_{76}O_{20}$.

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 0.010 g 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 Reference Standard in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under the Thin-layer Chromatography with the sample solution and the standard solution. Spot 20 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 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 $[\alpha]_D^{20}$: +32 - +35° (after drying, 0.5 g, methanol, 25 mL, 100 mm).

Loss on drying Not more than 7.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

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

Assay Weigh accurately about 0.05 g each of Lanatoside C and Lanatoside C Reference Standard, 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 the 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 the Ultraviolet-visible Spectrophotometry, using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

Amount (mg) of $C_{49}H_{76}O_{20}$
= amount (mg) of Lanatoside C Reference Standard
$\times \frac{A_T}{A_S}$

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

Lanatoside C Tablets

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Lanatoside C Tablets contain not less than 90%