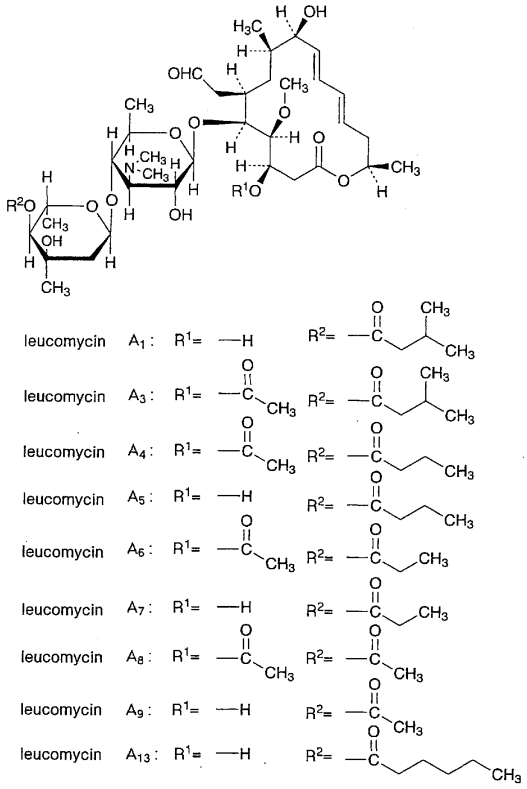
Kitasamycin

Leucomycin

キタサマイシン



(Leucomycins A_1, A_5, A_7, A_9, A_{13})
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[*O*-(4-*O*-Acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Leucomycin A_1 : acyl = 3-methylbutanoyl
Leucomycin A_5 : acyl = butanoyl
Leucomycin A_7 : acyl = propanoyl
Leucomycin A_9 : acyl = acetyl
Leucomycin A_{13}: acyl = hexanoyl

(Leucomycins A_3, A_4, A_6, A_8)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[*O*-(4-*O*-acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Leucomycin A_3: acyl = 3-methylbutanoyl
Leucomycin A_4: acyl = butanoyl
Leucomycin A_6: acyl = propanoyl
Leucomycin A_8: acyl = acetyl

[*1392-21-8*, Kitasamycin]

Kitasamycin contains not less than 900 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.93). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin occurs as a white to light yellow-white powder.

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

Identification Determine the absorption spectrum of a solution of Kitasamycin in methanol (1 in 40,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelength.

Content ratio of the active principle Dissolve 0.02 g of Kitasamycin in 20 mL of diluted acetonitrile (1 in 2), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 by the area percentage method: the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 are 40 to 70%, 5 to 25% and 3 to 12%, respectively. Relative retention times of leucomycin A_4 and leucomycin A_1 to that of leucomycin A_5 are 1.2 and 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

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

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

Mobile phase: To a volume of a solution of ammonium acetate (77 in 500) add diluted phosphoric acid (1 in 150) to adjust to pH 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of leucomycin A_5 is about 8 minutes.

Time span of measurement: About three times as long as the retention time of leucomycin A_5.

System suitability—

System performance: Dissolve about 0.02 g each of Kitasamycin Reference Standard and Josamycin Reference Standard in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A_5 and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak areas of leucomycin A_5 is not more than 1.0%.

Water 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 the Microbial Assay for Antibiotics according to the following conditions.

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

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

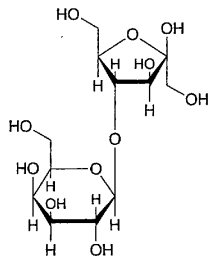
(3) Standard solutions—Weigh accurately an amount of Kitasamycin Reference Standard equivalent to about 0.03 g (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 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