amide 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—Prepare the test solution with 1.0 g of Acetohexamide according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(5) Related substances—Cyclohexylamine—To 0.20 g of Acetohexamide add exactly 2 mL of a mixture of *N,N*-dimethylformamide and acetone (1:1) to dissolve, and use this solution as the sample solution. Separately, dissolve 0.020 g of cyclohexylamine for thin-layer chromatography in a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate for 30 minutes or more. Then, develop the plate with a mixture of ethyl acetate, methanol, cyclohexane and ammonia water (28) (6:2:1:1) to a distance of about 10 cm, and heat the plate at 100°C for 10 minutes. Spray evenly ninhydrin-butanol TS on the plate, and heat at 120°C for 10 minutes: the spot from the sample solution corresponding to the spot obtained from the standard solution is not more intense than the spot from the standard solution.

Dicyclohexylurea—Dissolve 0.20 g of Acetohexamide in exactly 2 mL of a mixture of *N,N*-dimethylformamide and acetone (1:1), and use this solution as the sample solution. Separately, dissolve 0.020 g of dicyclohexylurea for thin-layer chromatography in a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of *N,N*-dimethylformamide and acetone (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate for 30 minutes or more. Develop the plate with a mixture of ethyl acetate, methanol, cyclohexane and ammonia water (28) (6:2:1:1) to a distance of about 10 cm, and heat the plate at 120°C for 10 minutes. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 120°C for 10 minutes: the spot from the sample solution corresponding to the spot obtained from the standard solution is not more intense than the spot from the standard solution.

Other related substances—Dissolve 0.10 g of Acetohexamide in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet two 1-mL portions of this solution, add acetone to make exactly 10 mL and 25 mL, respectively, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, cyclohexane and ammonia water (28) (6:2:1:1) 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 spot from the standard solution (1), and the number of them which are intense than the spot from the standard solution (2) is not more than 4.

Loss on drying Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.10% (1 g).

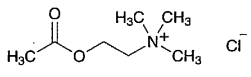
Assay Weigh accurately about 0.3 g of Acetohexamide, previously dried, dissolve in 30 mL of dimethylformamide, add 10 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination using a solution prepared by adding 19 mL of water to 30 mL of dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 32.440 mg of $C_{15}H_{20}N_2O_4S$

Containers and storage Containers—Well-closed containers.

Acetylcholine Chloride for Injection

注射用塩化アセチルコリン



$C_7H_{16}ClNO_2$: 181.66
N-(2-Acetoxyethyl)-*N,N,N*-trimethylammonium chloride
[*60-31-1*]

Acetylcholine Chloride for Injection is a preparation for injection which is dissolved before use. It contains not less than 98.0% and not more than 102.0% of acetylcholine chloride ($C_7H_{16}ClNO_2$), and not less than 19.3% and not more than 19.8% of chlorine (Cl: 35.45), calculated on the dried basis. It contains not less than 93% and not more than 107% of the labeled amount of acetylcholine chloride ($C_7H_{16}ClNO_2$).

Method of preparation Prepare as directed under Injections.

Description Acetylcholine Chloride for Injection occurs as white crystals or crystalline powder.

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

It is extremely hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Acetylcholine Chloride for Injection, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Acetylcholine Chloride for Injection (1 in 10) responds to the Qualitative Tests (2) for chloride.

Melting point 149 - 152°C. Seal Acetylcholine Chloride for Injection in a capillary tube for melting point immediately after drying both of the sample and the tube at 105°C for

3 hours, and determine the melting point.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Acetylcholine Chloride for Injection in 10 mL of water: the solution is clear and colorless.

(2) Acid—Dissolve 0.10 g of Acetylcholine Chloride for Injection in 10 mL of freshly boiled and cooled water, and add 1 drop of bromothymol blue TS, and 0.30 mL of 0.01 mol/L sodium hydroxide VS: the solution is blue in color.

(3) Heavy metals—Proceed with 2.0 g of Acetylcholine Chloride for Injection 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).

Loss on drying Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay **(1)** Acetylcholine chloride—Weigh accurately the contents of not less than 10 Acetylcholine Chloride for Injections. Weigh accurately about 0.5 g of the contents, dissolve in 15 mL of water, then add exactly 40 mL of 0.1 mol/L sodium hydroxide VS, stopper loosely, and heat on a water bath for 30 minutes. Cool quickly, and titrate the excess sodium hydroxide with 0.05 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.166 mg of $C_7H_{16}ClNO_2$

(2) Chlorine—Titrate the solution, which has been titrated in (1), with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

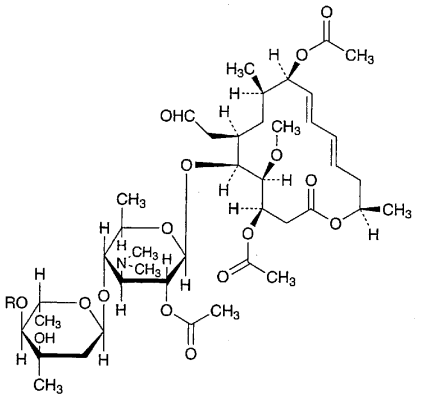
Each mL of 0.1 mol/L silver nitrate VS
= 3.5453 mg of Cl

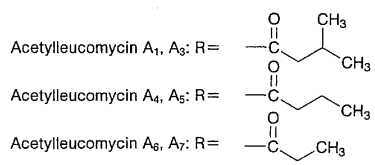
Containers and storage Containers—Hermetic containers.

Acetylkitasamycin

Acetylleucomycin

アセチルキタサマイシン





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

Acetylleucomycins A_1, A_3: acyl = 3-methylbutanoyl
Acetylleucomycins A_4, A_5: acyl = butanoyl
Acetylleucomycins A_6, A_7: acyl = propanoyl

Acetylkitasamycin contains not less than 560 μg (potency) per mg, calculated on the anhydrous basis. The potency of Acetylkitasamycin is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.94). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

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

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

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

(2) Determine the infrared absorption spectrum of Acetylkitasamycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water Not more than 5.0% (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 solution—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 0.1 mol/L 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 solution—Weigh accurately an amount of Acetylkitasamycin equivalent to about 0.03 g (potency), dissolve in 25 mL of methanol, add water to make exactly 50 mL, shake well, and allow to stand at 37 ± 2°C for 24 hours. 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 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution,