10 mL of a mixture of ethanol (95) and acetic acid (100) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of ethanol (95) and acetic acid (100) (9:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of ethanol (95) and acetic acid (100) (9:1) 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 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and acetic acid (100) (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 Not more than 0.4% (1 g, in vacuum, 60°C, 3 hours).

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

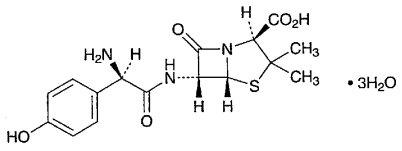
Assay Weigh accurately about 0.3 g of Amoxapine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.689 mg of $C_{17}H_{16}ClN_3O$

Containers and storage Containers—Tight containers.

Amoxicillin

アモキシシリン



$C_{16}H_{19}N_3O_5S.3H_2O$: 419.45
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)-acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid trihydrate [*61336-70-7*]

Amoxicillin contains not less than 750 μg (potency) per mg, calculated on the anhydrous basis. The potency of Amoxicillin is expressed as mass (potency) of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

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

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

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

Purity **(1)** Heavy metals—To 1.0 g of Amoxicillin add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), mix, and heat on a water bath to dryness. Carbonize the residue by gently heating. After cooling, add 1 mL of sulfuric acid, heat carefully, then heat at 500 - 600°C to incinerate. After cooling, add 1 mL of hydrochloric acid to the residue, and heat on a water bath to dryness. Then add 10 mL of water to the residue, and heat on a water bath to dissolve. After cooling, add ammonia TS to adjust the pH to 3 - 4, and add 2 mL of dilute acetic acid. If necessary, filter, wash the residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: To 2.0 mL of Standard Lead Solution add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), then proceed in the same manner as for preparation of the test solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Amoxicillin according to Method 4, and perform the test using Apparatus B (not more than 2 ppm).

Water Not less than 11.0% and not more than 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Amoxicillin and Amoxicillin Reference Standard, equivalent to about 0.1 g (potency), dissolve each in a solution of sodium tetraborate decahydrate (1 in 200) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the peak areas, A_T and A_S, of amoxicillin of each solution.

Amount [μg (potency)] of $C_{16}H_{19}N_3O_5S$
= amount [mg (potency)] of Amoxicillin Reference Standard $\times \frac{A_T}{A_S} \times 1000$

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 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

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

System suitability—

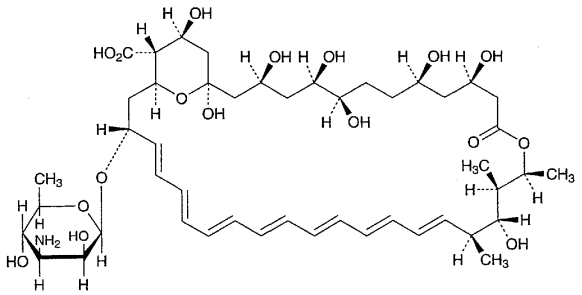
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical steps of the peak of amoxicillin is not less than 2500 steps.

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 amoxicillin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amphotericin B

アムホテリシン B



$C_{47}H_{73}NO_{17}$: 924.08
(1*R*,3*S*,5*R*,6*R*,9*R*, 11*R*, 15*S*, 16*R*, 17*R*, 18*S*, 19*E*, 21*E*, 23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*S*,37*S*)-33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [*1397-89-3*]

Amphotericin B contains not less than 840 μg (potency) per mg, calculated on the dried basis. The potency of Amphotericin B is expressed as mass (potency) of amphotericin B ($C_{47}H_{73}NO_{17}$).

Description Amphotericin B occurs as a yellow to orange powder.

It is freely soluble in dimethylsulfoxide and practically insoluble in water and in ethanol (95).

Identification (1) Dissolve 5 mg of Amphotericin B in 10 mL of dimethylsulfoxide. To 1 mL of this solution add 5 mL of phosphoric acid: a blue color develops between the two layers, and the solution becomes blue by shaking. After addition of 15 mL of water it becomes yellow to light yellow-brown by shaking.

(2) Dissolve 0.025 g of Amphotericin B in 5 mL of dimethylsulfoxide, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Amphotericin B Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelength.

Purity Amphotericin A—Weigh accurately about 0.05 g each of Amphotericin B and Amphotericin B Reference Standard, add exactly 10 mL each of dimethylsulfoxide to dissolve, and add methanol to make exactly 50 mL. Pipet 4 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and the standard solution (1), respectively. Separately, weigh accurately about 0.02 g of Nystatin Reference Standard, add exactly 40 mL of dimethylsulfoxide to dissolve, then add methanol to make exactly 200 mL. Pipet 4 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry using a solution obtained in the same manner as the sample solution as the blank, and determine the absorbances at 282 nm and at 304 nm. Calculate the amount of amphotericin A by the following equation: not more than 5% for Amphotericin B used for injections, and not more than 15% for Amphotericin B not used for injections.

$$\text{Amount (\%) of amphotericin A} = \left[\frac{\text{amount (mg) of Nystatin Reference Standard}}{\text{amount (mg) of the sample}}\right] \times \frac{[A_{Sa1} \times A_{T2}) - (A_{Sa2} \times A_{T1})]}{[(A_{Sa1} \times A_{Sb2}) - (A_{Sa2} \times A_{Sb1})]} \times 25$$

A_{Sb1}: Absorbance at 282 nm of the standard solution (2)
A_{Sa1}: Absorbance at 282 nm of the standard solution (1)
A_{Sb2}: Absorbance at 304 nm of the standard solution (2)
A_{Sa2}: Absorbance at 304 nm of the standard solution (1)
A_{T1}: Absorbance at 282 nm of the sample solution
A_{T2}: Absorbance at 304 nm of the sample solution

Loss on drying Not more than 5.0% (0.1 g, in vacuum, 60°C, 3 hours).

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—*Saccharomyces cerevisiae* ATCC 9763

(2) Culture medium—Use the medium 2) Medium for test organism [12] under (1) Agar media for seed and base layer.

(3) Preparation of cylinder-agar plate—Proceed as directed in 5 under the Cylinder plate method, using Petri dish plates not dispensing the agar medium for base layer and dispensing 8.0 mL of the seeded agar medium.

(4) Standard solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B Reference Standard equivalent to about 0.02 g (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, and add dimethylsulfoxide to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(5) Sample solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B equivalent to about 0.02 g (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.