Detection sensitivity: Adjust the detection sensitivity so that the peak height of fosfestrol obtained from 10 μL of the standard solution is between 5 mm and 15 mm.

Time span of measurement: Three times as long as the retention time of fosfestrol.

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

Assay Weigh accurately about 0.2 g of Fosfestrol, previously dried, dissolve in 60 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). The end point is the second equivalent point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 10.708 mg of $C_{18}H_{22}O_8P_2$

Containers and storage Containers—Tight containers.

Fosfestrol Tablets

Diethylstilbestrol Diphosphate Tablets

ホスフェストロール錠

Fosfestrol Tablets contain not less than 93% and not more than 107% of the labeled amount of fosfestrol ($C_{18}H_{22}O_8P_2$: 428.31).

Method of preparation Prepare as directed under the Tablets, with Fosfestrol.

Identification **(1)** To a quantity of powdered Fosfestrol Tablets, equivalent to 0.5 g of Fosfestrol according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, and filter. To the filtrate add 100 mL of diethyl ether, extract, and evaporate carefully the diethyl ether extract on a water bath to dryness. Proceed with 0.015 g of the residue as directed in the Identification (1) under Fosfestrol.

(2) Dry 0.01 g of the residue obtained in (1) at 105°C for 4 hours, and determine the infrared absorption spectrum as directed in the potassium bromide disk method under the Infrared Spectrometry: it exhibits absorption at the wave numbers of about 2970 cm^{-1}, 1605 cm^{-1}, 1505 cm^{-1}, 1207 cm^{-1} and 1006 cm^{-1}.

Dissolution test Perform the test with 1 tablet of Fosfestrol Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water. Take 20 mL or more of the dissolved solution 20 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent, add a solution of sodium hydroxide (1 in 250) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of Fosfestrol Reference Standard, previously dried at 105°C for 4 hours, and dissolve in a solution of sodium hydroxide (1 in 250) to make exactly 100 mL. Pipet 2 mL of this solution, add a solution of sodium hydroxide (1 in 250) 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 242 nm as directed under the Ultraviolet-visible Spectrophotometry.

The dissolution rate of Fosfestrol Tablets in 20 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of fosfestrol ($C_{18}H_{22}O_8P_2$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{1}{C} \times 180$$

W_S: Amount (mg) of Fosfestrol Reference Standard.
C: Labeled amount (mg) of fosfestrol ($C_{18}H_{22}O_8P_2$) in 1 tablet.

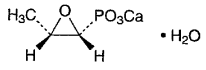
Assay Weigh accurately not less than 20 Fosfestrol Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 1 g of fosfestrol ($C_{18}H_{22}O_8P_2$) according to the labeled amount, add 100 mL of a solution of sodium hydroxide (1 in 125), shake well, add water to make exactly 500 mL. Filter this solution, discard the first 30 mL of the filtrate, pipet the subsequent 2 mL of the filtrate, add 30 mL of a solution of sodium hydroxide (1 in 125) and water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.08 g of Fosfestrol Reference Standard, previously dried at 105°C for 4 hours, and dissolve in a solution of sodium hydroxide (1 in 125) to make exactly 50 mL. Pipet 1 mL of this solution, add 10 mL of a solution of sodium hydroxide (1 in 125) 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 the standard solution at 242 nm as directed under the Ultraviolet-visible Spectrophotometry.

Amount (mg) of fosfestrol ($C_{18}H_{22}O_8P_2$)
= amount (mg) of Fosfestrol Reference Standard
$\times \frac{A_T}{A_S} \times \frac{25}{2}$

Containers and storage Containers—Tight containers.

Fosfomycin Calcium

ホスホマイシンカルシウム



$C_3H_5CaO_4P.H_2O$: 194.14
Monocalcium (2*R*,3*S*)-3-methyloxiran-2-ylphosphonate monohydrate [*26016-98-8*]

Fosfomycin Calcium contains not less than 725 μg (potency) per mg, calculated on the anhydrous basis. The potency of Fosfomycin Calcium is expressed as mass (potency) of fosfomycin ($C_3H_7O_4P$: 138.06).

Description Fosfomycin Calcium occurs as a white crystalline powder.

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

Identification **(1)** Determine the infrared absorption spectrum of Fosfomycin Calcium 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) Determine the spectrum of a solution of Fosfomycin Calcium in heavy water for nuclear magnetic resonance spectroscopy (1 in 300), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H): it exhibits sharp single signals, at around δ 1.50 ppm, δ 2.87 ppm and δ 3.33 ppm, respectively, and exhibit no signal at around δ 1.36 ppm.

(3) A solution of Fosfomycin Calcium (1 in 500) responds to the Qualitative Test (3) for calcium salt.

Optical rotation $[\alpha]_D^{20}$: -2.5 – $-5.4°$ (0.50 g calculated on the anhydrous bases, 0.4 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, pH 8.5, 10 mL, 100 mm).

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Calcium, add 40 mL of sodium periodate (107 in 10,000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 0.07 g of potassium dihydrogenphosphate, proceed with this solution in the same manner as directed for the preparation of the sample stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the sample stock solution without using Fosfomycin Calcium, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the sample stock solution, the standard stock solution, and the blank stock solution, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, mix, and add water to make 25 mL, and use these solutions as the sample solution, the standard solution, and the blank solution, respectively. After allowing these solutions to stand for 30 minutes at 20 ± 1°C, perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry, using water as a blank, and determine the absorbances at 740 nm, A_T, A_S and A_B, of the sample solution, the standard solution and the blank solution: the content of phosphorus is 15.2 – 16.7%.

Amount (mg) of phosphorus (P)
= amount (mg) of potassium dihydrogenphosphate
$\times \dfrac{A_T - A_B}{A_S - A_B} \times 0.22760$

Calcium Content Weigh accurately about 0.2 g of Fosfomycin Calcium, add 4 mL of 1 mol/L Hydrochloric acid TS, and shake well until the sample is completely dissolved. To this solution add 100 mL of water, 9 mL of sodium hydroxide TS and 0.1 g of methylthymol blue-sodium chloride indicator, and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from clear blue to gray or gray-purple: calcium content is 19.6 – 21.7%. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.0040 mg of Ca

Purity (1) Heavy metals—Proceed with 1.0 g of Fosfomycin Calcium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

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

Water Not more than 12.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

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—*Proteus* sp. (MB838)

(2) Culture medium—Mix 5.0 g of peptone, 3.0 g of meat extract, 2.0 g of yeast extract, and 15 g of agar in 1000 mL of water, sterilize, and use as the agar media for base layer and seed layer with the pH of between 6.5 and 6.6 after sterilization.

(3) Seeded agar layer—Incubate the test organism on the slant of the agar medium for transferring test organisms at 37°C for 40 – 48 hours. Subcultures at least three times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organisms in a Roux bottle, incubate at 37°C for 40 – 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water, and use this as the stock suspension of test organism. The amount of the water to be added is adjust so that the percent transmission at 560 nm of the suspension diluted ten times with water is 17%. Keep the stock suspension at 10°C or below and use within 7 days. Add 1.0 – 2.0 mL of the stock suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the deeded agar layer.

(4) Standard solution—Weigh accurately an amount of Fosfomycin Phenethylammonium Reference Standard equivalent to about 0.02 g (potency), dissolve in 0.05 mol/L Tris buffer solution, pH 7.0 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 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(5) Sample solution—Weigh accurately an amount of Fosfomycin Calcium equivalent to about 0.02 g (potency), and dissolve in 0.05 mol/L Tris buffer solution, pH 7.0 to make exactly 50 mL. To exactly a suitable amount of this solution add 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 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.