of *sodium pyrophosphate R* and dilute to 1000 ml with *water R* (solution B). Mix about 700 ml of solution A and about 300 ml of solution B to obtain a solution of pH 4.4. To 1000 ml of this solution, add 0.25 g of *tetrahexylammonium hydrogen sulphate R* and 100 ml of *methanol R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 230 nm.

Injection: 20 µl.

Run time: 2.5 times the retention time of foscarnet.

System suitability: reference solution (b):

– *resolution*: minimum 7 between the peaks due to foscarnet and impurity B.

Limits:

– *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent);
– *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.04 per cent); disregard any peak with a relative retention time less than 0.6.

Phosphate and phosphite. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 60.0 mg of the substance to be examined in *water R* and dilute to 25.0 ml with the same solvent.

Reference solution (a). Dissolve 28 mg of *sodium dihydrogen phosphate monohydrate R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (b). Dissolve 43 mg of *sodium phosphite pentahydrate R* in *water R* and dilute to 100 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 25 ml with *water R*.

Reference solution (d). Dilute 3 ml of reference solution (a) and 3 ml of reference solution (b) to 25 ml with *water R*.

Column:

– *size*: *l* = 0.05 m, Ø = 4.6 mm;
– *stationary phase*: *anion exchange resin R*.

Mobile phase: dissolve 0.102 g of *potassium hydrogen phthalate R* in *water R*, add 2.5 ml of *1 M nitric acid* and dilute to 1000 ml with *water R*.

Flow rate: 1.4 ml/min.

Detection: spectrophotometer at 290 nm (indirect detection).

Injection: 20 µl of the test solution and reference solutions (c) and (d).

System suitability: reference solution (d):

– *resolution*: minimum 2.0 between the peaks due to phosphate (1st peak) and phosphite;
– *signal-to-noise ratio*: minimum 10 for the principal peak.

Limits:

– *phosphate*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
– *phosphite*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent).

Heavy metals: maximum 10 ppm.

Dissolve 1.25 g in 12.5 ml of *1 M hydrochloric acid*. Warm on a water-bath for 3 min and cool to room temperature. Transfer to a beaker and adjust the pH to about 3.5 with *dilute ammonia R1* and dilute to 25 ml with *water R* (solution A). To 12 ml of solution A, add 2.0 ml of *buffer solution pH 3.5 R*. Rapidly pour the mixture into a test tube containing 1 drop of *sodium sulphide solution R*. The solution is not more intensely coloured than a reference solution prepared simultaneously and in the same manner pouring a mixture of 5.0 ml of *lead standard solution (1 ppm Pb) R*, 5.0 ml of *water R*, 2.0 ml of solution A and 2.0 ml of *buffer solution pH 3.5 R* into a test tube containing 1 drop of *sodium sulphide solution R*.

Loss on drying (*2.2.32*): 35.0 per cent to 37.0 per cent, determined on 1.000 g by drying in an oven at 150 °C.

Bacterial endotoxins (*2.6.14*): less than 83.3 IU/g, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Dissolve 0.200 g in 50 ml of *water R*. Titrate with *0.05 M sulphuric acid*, determining the end-point potentiometrically (*2.2.20*) at the 1st inflexion point.

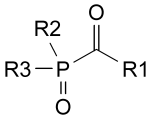
1 ml of *0.05 M sulphuric acid* is equivalent to 19.20 mg of CNa_3O_5P.

STORAGE

Protected from light.

IMPURITIES

Specified impurities: A, B, C, D.



A. R1 = OC_2H_5, R2 = R3 = ONa: disodium (ethoxycarbonyl)phosphonate,

B. R1 = R2 = ONa, R3 = OC_2H_5: disodium (ethoxyoxydophosphanyl)formate,

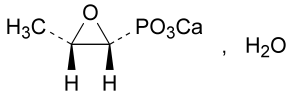
C. R1 = R2 = OC_2H_5, R3 = ONa: ethyl sodium (ethoxycarbonyl)phosphonate,

D. R1 = R2 = R3 = OC_2H_5: methyl (diethoxyphosphoryl)-formate.

01/2008:1328
corrected 6.0

FOSFOMYCIN CALCIUM

Fosfomycinum calcicum



$C_3H_5CaO_4P,H_2O$ M_r 194.1

DEFINITION

Calcium (2*R*,3*S*)-(3-methyloxiran-2-yl)phosphonate monohydrate.

Substance produced by certain strains of *Streptomyces fradiae* or obtained by any other means.

Content: 95.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: slightly soluble in water, practically insoluble in acetone, in methanol and in methylene chloride.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs of *potassium bromide R*.

Comparison: *Ph. Eur. reference spectrum of fosfomycin calcium*.

B. Dissolve about 0.1 g in 3 ml of a 25 per cent *V/V* solution of *perchloric acid R*. Add 1 ml of *0.1 M sodium periodate* and heat on a water-bath for 30 min. Allow to cool and add 50 ml of *water R*. Neutralise with a saturated solution of *sodium hydrogen carbonate R* and add 1 ml of a freshly prepared 400 g/l solution of *potassium iodide R*. Prepare a blank at the same time and in the same manner. The test solution remains colourless and the blank is orange.

C. To about 8 mg add 2 ml of *water R*, 1 ml of *perchloric acid R* and 2 ml of *0.1 M sodium periodate*. Heat on a water-bath for 10 min and add, without cooling, 1 ml of *ammonium molybdate solution R5* and 1 ml of *aminohydroxynaphthalenesulphonic acid solution R*. Allow to stand for 30 min. A blue colour develops.

D. It gives reaction (a) of calcium (*2.3.1*).

TESTS

pH (*2.2.3*): 8.1 to 9.6.

Dissolve 20 mg in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

Specific optical rotation (*2.2.7*): – 11.0 to – 13.0 (anhydrous substance).

Dissolve 2.5 g in a 125 g/l solution of *sodium edetate R* previously adjusted to pH 8.5 with *strong sodium hydroxide solution R*, and dilute to 50.0 ml with the same solution. Measure at 405 nm using a mercury lamp.

Impurity A: maximum 1.5 per cent.

In a glass-stoppered flask, dissolve 0.200 g in 100.0 ml of *water R*. Add 50 ml of *0.5 M phthalate buffer solution pH 6.4 R* and 5.0 ml of *0.005 M sodium periodate*, close and shake. Allow to stand protected from light for 90 min. Add 10 ml of a freshly prepared 400 g/l solution of *potassium iodide R*, close and shake for 2 min. Titrate with *0.0025 M sodium arsenite* until the yellow colour almost disappears. Add 2 ml of *starch solution R* and slowly continue the titration until the colour is completely discharged. Carry out a blank test under the same conditions. Calculate the percentage content of $C_3H_7CaO_5P$ using the following expression:

$$\frac{(n_1 - n_2) \times c \times 97}{m\,(100 - H)} \times 100$$

m = mass of the substance to be examined, in milligrams;

n_1 = volume of *0.0025 M sodium arsenite* used in the blank titration;

n_2 = volume of *0.0025 M sodium arsenite* used in the titration of the test solution;

c = molarity of the sodium arsenite solution;

H = percentage content of water.

Chlorides (*2.4.4*): maximum 0.2 per cent.

Dissolve 0.500 g in *water R*, add 2 ml of *nitric acid R* and dilute to 50 ml with the same acid. To 2.5 ml of this solution add 12.5 ml of *water R*.

Heavy metals (*2.4.8*): maximum 20 ppm.

Dissolve 2.5 g in 6 ml of *glacial acetic acid R* and dilute to 25.0 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (2 ppm Pb) R*.

Water (*2.5.12*): 8.5 per cent to 11.5 per cent, determined on 0.250 g. Use as the solvent a mixture of 1 volume of *pyridine R* and 3 volumes of *ethylene glycol R*.

ASSAY

In a glass-stoppered flask, dissolve 0.120 g in 20.0 ml of *0.1 M sodium periodate*. Add 5 ml of a 50 per cent *V/V* solution of *perchloric acid R* and shake. Heat in a water-bath at 37 °C for 105 min. Add 50 ml of *water R* and immediately adjust to pH 6.4 with a saturated solution of *sodium hydrogen carbonate R*. Add 10 ml of a freshly prepared 400 g/l solution of *potassium iodide R*, close and allow to stand for 2 min. Titrate with *0.1 M sodium arsenite* until the yellow colour almost disappears. Add 2 ml of *starch solution R* and slowly continue the titration until the colour is completely discharged. Carry out a blank test under the same conditions.

Calculate the percentage content of $C_3H_5CaO_4P$ using the following expression:

$$\frac{(n_1 - n_2) \times c \times 88 \times 100}{m\,(100 - H)} \times 100 - G$$

m = mass of the substance to be examined, in milligrams;

n_1 = volume of *0.1 M sodium arsenite* used in the blank titration;

n_2 = volume of *0.1 M sodium arsenite* used in the titration of the test solution;

c = molarity of the sodium arsenite solution;

G = percentage content of impurity A;

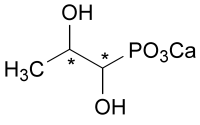
H = percentage content of water.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities: *A*.



A. calcium (1,2-dihydroxypropyl)phosphonate.