Chlorides (*2.4.4*): maximum 200 ppm.

To 0.5 g add a mixture of 0.2 mL of *nitric acid R* and 30 mL of *water R* and shake for 5 min. Allow to stand for 15 min and filter.

Sulfates (*2.4.13*): maximum 300 ppm.

To 1.0 g add a mixture of 0.2 mL of *acetic acid R* and 30 mL of *distilled water R* and shake for 5 min. Allow to stand for 15 min and filter.

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

1.0 g complies with test C. Prepare the reference solution using 2 mL of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 20 mL of *dimethylformamide R*. Titrate with *0.1 M sodium hydroxide* using 0.2 mL of *bromothymol blue solution R2*. Carry out a blank titration.

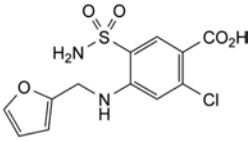
1 mL of *0.1 M sodium hydroxide* is equivalent to 33.07 mg of $C_{12}H_{11}ClN_2O_5S$.

STORAGE

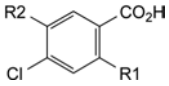
Protected from light.

IMPURITIES

Specified impurities: A, B, C, D, E.



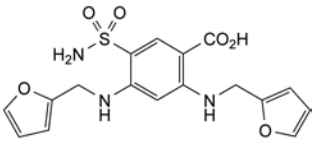
A. 2-chloro-4-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid,



B. R1 = Cl, R2 = SO_2-NH_2: 2,4-dichloro-5-sulfamoylbenzoic acid,

C. R1 = NH_2, R2 = SO_2-NH_2: 2-amino-4-chloro-5-sulfamoylbenzoic acid,

E. R1 = Cl, R2 = H: 2,4-dichlorobenzoic acid,



D. 2,4-bis[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid.

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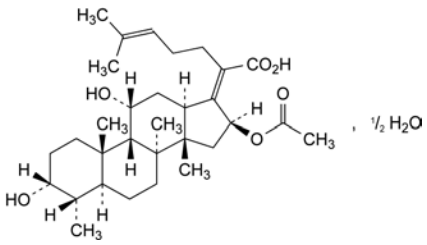
FUSIDIC ACID

Acidum fusidicum



$C_{31}H_{48}O_6,{}^1/_2H_2O$ M_r 525.7

DEFINITION

ent-(17*Z*)-16α-(Acetyloxy)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid hemihydrate.

Antimicrobial substance produced by the growth of certain strains of *Fusidium coccineum* or by any other means.

Content: 97.5 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of fusidic acid.*

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution. Dissolve 24 mg of *diethanolamine fusidate CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *methanol R, cyclohexane R, glacial acetic acid R, chloroform R* (2.5:10:10:80 *V/V/V/V*).

Application: 10 µL.

Development: over a path of 15 cm.

Drying: in a current of warm air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 50 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a). Dissolve 5 mg of *3-ketofusidic acid CRS* in 5 mL of the mobile phase. To 1.0 mL of this solution add 0.20 mL of the test solution and dilute to 20.0 mL with the mobile phase.

Reference solution (b). Dilute 20 µL of the test solution to 100.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.125-0.15 m, Ø = 4-5 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *methanol R*, 10 g/L solution of *phosphoric acid R, water R, acetonitrile R* (10:20:20:50 *V/V/V/V*).

Flow rate: 2 mL/min.

Detection: spectrophotometer at 235 nm.

Injection: 20 µL.

Run time: 3.5 times the retention time of fusidic acid.

System suitability:

– *resolution*: minimum 2.5 between the peaks due to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with reference solution (a);
– *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

– *total*: not more than twice the area of the peak due to fusidic acid in the chromatogram obtained with reference solution (a) (2.0 per cent);
– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Water (*2.5.12*): 1.4 per cent to 2.0 per cent, determined on 0.50 g.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.500 g in 10 mL of *ethanol (96 per cent) R*. Add 0.5 mL of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 mL of *0.1 M sodium hydroxide* is equivalent to 51.67 mg of $C_{31}H_{48}O_6$.

STORAGE

Protected from light, at a temperature of 2 °C to 8 °C.