B. Dissolve 0.1 g in 10 mL of *water R*. Add 3 mL of *cupri-tartaric solution R* and heat. A red precipitate is formed.

C. To 1 mL of solution S (see Tests) add 9 mL of *water R*. To 1 mL of the solution add 5 mL of *hydrochloric acid R* and heat to 70 °C. A brown colour develops.

D. Dissolve 5 g in *water R* and dilute to 10 mL with the same solvent. To 0.5 mL of the solution add 0.2 g of *resorcinol R* and 9 mL of *dilute hydrochloric acid R* and heat on a water-bath for 2 min. A red colour develops.

TESTS

Solution S. Dissolve 10.0 g in *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution. Dissolve 5.0 g in *water R* and dilute to 10 mL with the same solvent. The solution is clear (*2.2.1*). Add 10 mL of *water R*. The solution is colourless (*2.2.2, Method II*).

Acidity or alkalinity. Dissolve 6.0 g in 25 mL of *carbon dioxide-free water R* and add 0.3 mL of *phenolphthalein solution R*. The solution is colourless. Not more than 0.15 mL of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*): – 91.0 to – 93.5 (anhydrous substance).

Dissolve 10.0 g in 80 mL of *water R*, add 0.2 mL of *dilute ammonia R1*, allow to stand for 30 min and dilute to 100.0 mL with *water R*.

Foreign sugars. Dissolve 5.0 g in *water R* and dilute to 10 mL with the same solvent. To 1 mL of the solution add 9 mL of *ethanol (96 per cent) R*. Any opalescence in the solution is not more intense than that in a mixture of 1 mL of the initial solution and 9 mL of *water R*.

5-Hydroxymethylfurfural and related compounds. To 5 mL of solution S add 5 mL of *water R*. The absorbance (*2.2.25*) measured at 284 nm is not greater than 0.32.

Barium. To 10 mL of solution S add 1 mL of *dilute sulfuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 mL of *distilled water R* and 10 mL of solution S.

Lead (*2.4.10*): maximum 0.5 ppm.

Water (*2.5.12*): maximum 0.5 per cent, determined on 1.00 g.

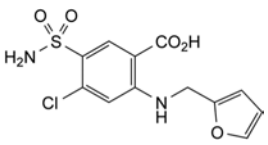
Sulfated ash : maximum 0.1 per cent.

Dissolve 5.0 g in 10 mL of *water R*, add 2 mL of *sulfuric acid R*, evaporate to dryness on a water-bath and ignite to constant mass.

01/2008:0391
corrected 6.0

FUROSEMIDE

Furosemidum



$C_{12}H_{11}ClN_2O_5S$ M_r 330.7
[54-31-9]

DEFINITION

4-Chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

mp: about 210 °C, with decomposition.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 50 mg in a 4 g/L solution of *sodium hydroxide R* and dilute to 100 mL with the same solution. Dilute 1 mL of this solution to 100 mL with a 4 g/L solution of *sodium hydroxide R*.

Spectral range: 220-350 nm.

Absorption maxima: at 228 nm, 270 nm and 333 nm.

Absorbance ratio: A_{270} / A_{228} = 0.52 to 0.57.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *furosemide CRS*.

C. Dissolve about 25 mg in 10 mL of *ethanol (96 per cent) R*. To 5 mL of this solution add 10 mL of *water R*. To 0.2 mL of the solution add 10 mL of *dilute hydrochloric acid R* and heat under a reflux condenser for 15 min. Allow to cool and add 18 mL of *1 M sodium hydroxide* and 1 mL of a 5 g/L solution of *sodium nitrite R*. Allow to stand for 3 min, add 2 mL of a 25 g/L solution of *sulfamic acid R* and mix. Add 1 mL of a 5 g/L solution of *naphthylethylenediamine dihydrochloride R*. A violet-red colour develops.

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use and protect from light.*

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a). Dissolve 2.0 mg of *furosemide impurity A CRS* in the mobile phase and dilute to 2.0 mL with the mobile phase.

Reference solution (b). Dilute a mixture of 1.0 mL of the test solution and 1.0 mL of reference solution (a) to 20.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: dissolve 0.2 g of *potassium dihydrogen phosphate R* and 0.25 g of *cetrimide R* in 70 mL of *water R*; adjust to pH 7.0 with *ammonia R* and add 30 mL of *propanol R*.

Flow rate: 1 mL/min.

Detection: spectrophotometer at 238 nm.

Injection: 20 µL of the test solution and reference solution (b).

Run time: 3 times the retention time of furosemide.

System suitability: reference solution (b):

– *resolution*: minimum 4 between the peaks due to impurity A (1st peak) and furosemide (2nd peak).

Limits:

– *impurities A, B, C, D, E*: for each impurity, not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) (0.25 per cent);

– *total*: not more than twice the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) (0.5 per cent);

– *disregard limit*: 0.1 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (b) (0.025 per cent).

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.

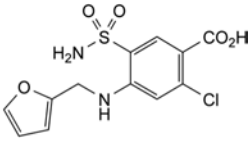
1 mL of *0.1 M sodium hydroxide* is equivalent to 33.07 mg of $C_{12}H_{11}ClN_2O_5S$.

STORAGE

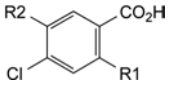
Protected from light.

IMPURITIES

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



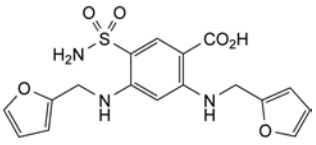
A. 2-chloro-4-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid,



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,

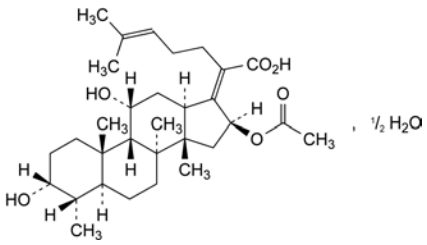


D. 2,4-bis[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid.

01/2008:0798

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.