for peak identification CRS and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C and D.

Relative retention with reference to furosemide (retention time = about 9 min): impurity C = about 0.5; impurity A = about 0.8; impurity D = about 1.5.

System suitability:

- *resolution*: minimum 4.0 between the peaks due to impurity A and furosemide in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 40 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 1.4; impurity D = 2.0;
- *impurity C*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 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.

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 40 mL of *dimethylformamide R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

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

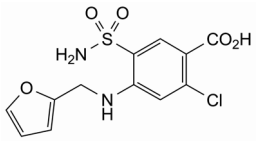
STORAGE

Protected from light.

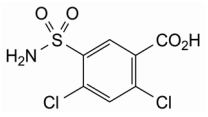
IMPURITIES

Specified impurities: C, D.

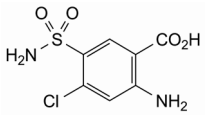
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, E, F.*



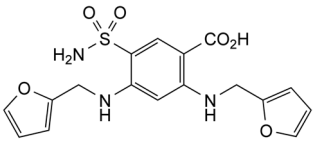
A. 2-chloro-4-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid,



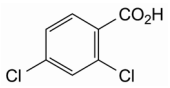
B. 2,4-dichloro-5-sulfamoylbenzoic acid,



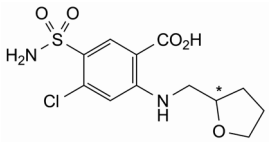
C. 2-amino-4-chloro-5-sulfamoylbenzoic acid,



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



E. 2,4-dichlorobenzoic acid,

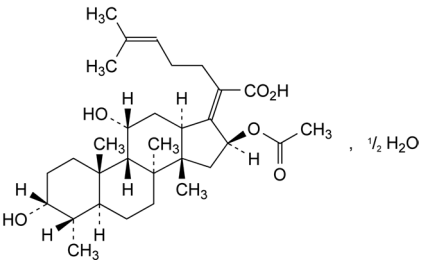


F. 4-chloro-5-sulfamoyl-2-[[((2*RS*)-tetrahydrofuran-2-yl)methyl]amino]benzoic acid.

01/2012:0798

FUSIDIC ACID

Acidum fusidicum



$C_{31}H_{48}O_6,½H_2O$ M_r 525.7

[6990-06-3]

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 fermentation 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: *fusidic acid CRS*.

B. Ignite 1 g. The residue does not give reaction (a) of sodium (*2.3.1*).

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture: *methanol R*, 5 g/L solution of *phosphoric acid R*, *acetonitrile R* (10:40:50 *V/V/V*).

Test solution. Dissolve 25 mg of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solution (a). Dissolve 2 mg of *fusidic acid for peak identification CRS* (containing impurities A, B, C, D, F, G, H and N) in the solvent mixture and dilute to 1.0 mL with the solvent mixture.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture.

Reference solution (c). Dilute 1.0 mL of reference solution (b) to 10.0 mL with the solvent mixture.

Reference solution (d). Dissolve the contents of a vial of *fusidic acid impurity mixture CRS* (containing impurities I, K, L and M) in 1.0 mL of the solvent mixture.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: *methanol R*, *acetonitrile R*, 5 g/L solution of *phosphoric acid R* (20:40:40 *V/V/V*);
- *mobile phase B*: 5 g/L solution of *phosphoric acid R*, *methanol R*, *acetonitrile R* (10:20:70 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 28	100 → 0	0 → 100
28 - 33	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 235 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *fusidic acid for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D, F, G, H and N; use the chromatogram supplied with *fusidic acid impurity mixture CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities I, K, L and M.

Relative retention with reference to fusidic acid (retention time = about 18 min): impurity A = about 0.4; impurity B = about 0.5; impurity C = about 0.6; impurity D = about 0.63; impurity N = about 0.65; impurity F = about 0.7; impurity G = about 0.82; impurity H = about 0.85; impurity I = about 0.96; impurity K = about 1.18; impurity L = about 1.23; impurity M = about 1.4.

System suitability: reference solution (a):

- *resolution*: minimum 1.5 between the peaks due to impurities G and H.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity C = 0.7; impurity D = 0.7; impurity F = 0.3; impurity I = 0.6; impurity K = 0.6;
- *impurity M*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
- *impurity G*: not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
- *impurity L*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *impurity B*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.4 per cent);
- *impurity A*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
- *impurities C, D, F, I, K, N*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
- *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 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 0.400 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 $C_{31}H_{48}O_6$.

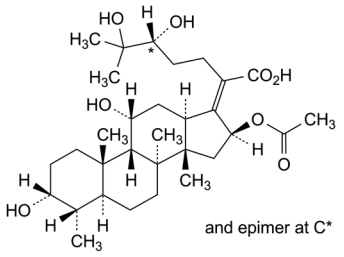
STORAGE

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

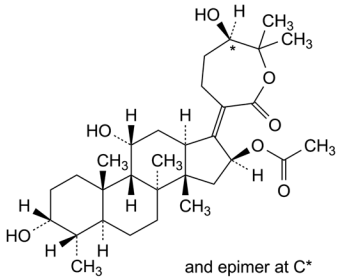
IMPURITIES

Specified impurities: A, B, C, D, F, G, I, K, L, M, N.

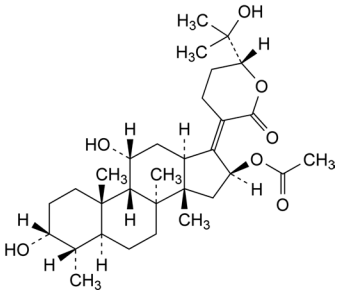
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *E, H, J, O.*



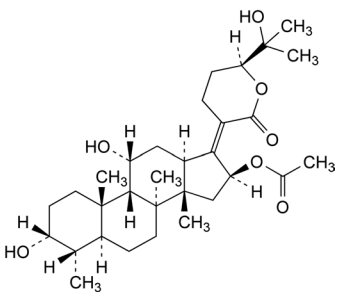
A. *ent*-(24*SR*,17*Z*)-16α-(acetyloxy)-3β,11β,24,25-tetrahydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholest-17(20)-en-21-oic acid (24,25-dihydro-24,25-dihydroxyfusidic acid),



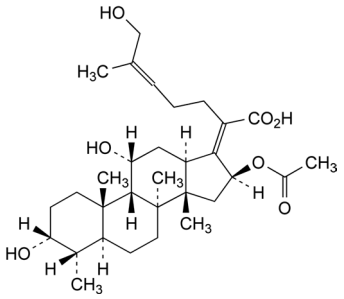
B. *ent*-(17*Z*)-3β,11β-dihydroxy-17-[(6*SR*)-6-hydroxy-7,7-dimethyl-2-oxooxepan-3-ylidene]-4β,8,14-trimethyl-18-nor-5β,10α-androstan-16α-yl acetate (24,25-dihydro-24,25-dihydroxyfusidic acid 21,25-lactone),



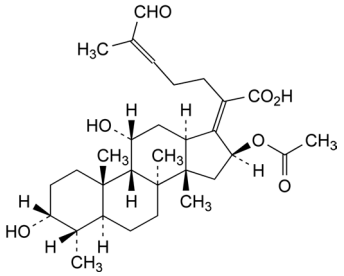
C. *ent*-(17*Z*)-3β,11β-dihydroxy-17-[(6*S*)-6-(1-hydroxy-1-methylethyl)-2-oxodihydro-2*H*-pyran-3(4*H*)-ylidene]-4β,8,14-trimethyl-18-nor-5β,10α-androstan-16α-yl acetate ((24*R*)-24,25-dihydro-24,25-dihydroxyfusidic acid 21,24-lactone),



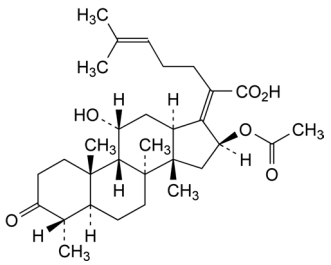
D. *ent*-(17*Z*)-3β,11β-dihydroxy-17-[(6*R*)-6-(1-hydroxy-1-methylethyl)-2-oxodihydro-2*H*-pyran-3(4*H*)-ylidene]-4β,8,14-trimethyl-18-nor-5β,10α-androstan-16α-yl acetate ((24*S*)-24,25-dihydro-24,25-dihydroxyfusidic acid 21,24-lactone),



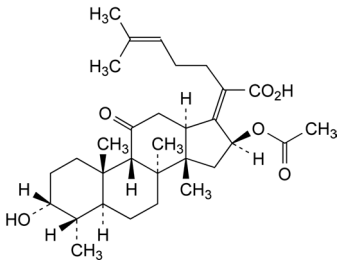
E. *ent*-(17*Z*,24*EZ*)-16α-(acetyloxy)-3β,11β,26-trihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (26-hydroxyfusidic acid),



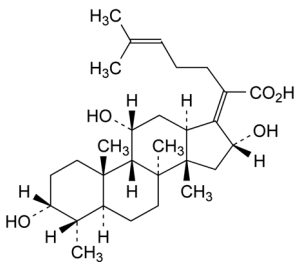
F. *ent*-(17*Z*,24*EZ*)-16α-(acetyloxy)-3β,11β-dihydroxy-4β,8,14-trimethyl-26-oxo-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (26-oxofusidic acid),



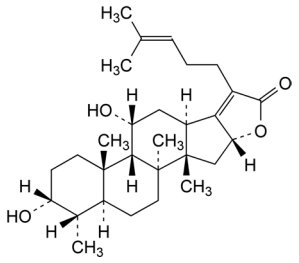
G. *ent*-(17*Z*)-16α-(acetyloxy)-11β-hydroxy-4β,8,14-trimethyl-3-oxo-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (3-didehydrofusidic acid),



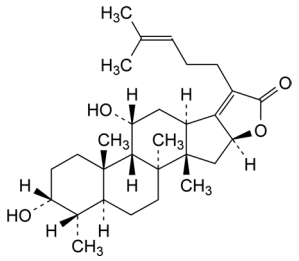
H. *ent*-(17*Z*)-16α-(acetyloxy)-3β-hydroxy-4β,8,14-trimethyl-11-oxo-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (11-didehydrofusidic acid),



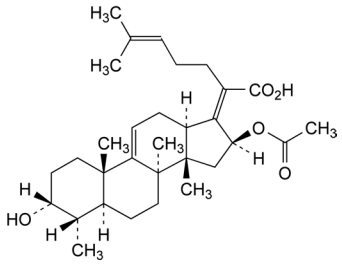
I. *ent*-(17*Z*)-3β,11β,16β-trihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid (16-*epi*-deacetylfusidic acid),



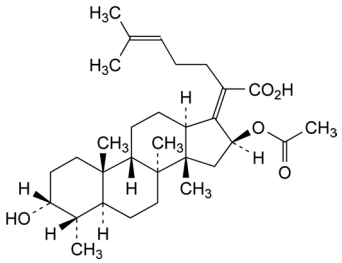
J. *ent*-(17*Z*)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dieno-21(16β)-lactone (16-*epi*-deacetylfusidic acid 21,16-lactone),



K. *ent*-(17*Z*)-3β,11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dieno-21(16α)-lactone (deacetylfusidic acid 21,16-lactone),

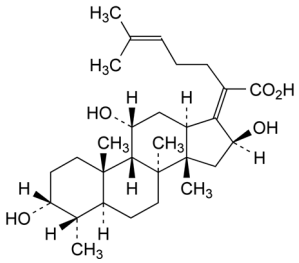


L. *ent*-(17*Z*)-16α-(acetyloxy)-3β-hydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-9(11),17(20),24-trien-21-oic acid (9,11-anhydrofusidic acid),



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

N. unknown structure,



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