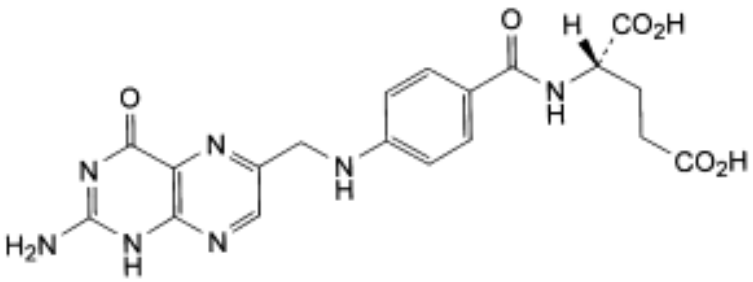
Folic Acid

General Notices

(Ph Eur monograph 0067)



$C_{19}H_{19}N_7O_6$ *441.4* 59-30-3

Action and use

Used in prevention of neural tube defects and treatment and prevention of megaloblastic anaemias.

Preparations

Folic Acid Tablets

Ferrous Fumarate and Folic Acid Tablets

Ph Eur

DEFINITION

(2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid.

Content

96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellowish or orange, crystalline powder.

Solubility

Practically insoluble in water and in most organic solvents. It dissolves in dilute acids and in alkaline solutions.

IDENTIFICATION

First identification *A, B.*

Second identification *A, C.*

A. Specific optical rotation *(2.2.7)*: + 18 to + 22 (anhydrous substance).

Dissolve 0.25 g in *0.1 M sodium hydroxide* and dilute to 25.0 ml with the same solvent.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

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

Test solution Dissolve 50 mg of the substance to be examined in a mixture of 2 volumes of *concentrated ammonia R* and 9 volumes of *methanol R* and dilute to 100 ml with the same mixture of solvents.

Reference solution Dissolve 50 mg of *folic acid CRS* in a mixture of 2 volumes of *concentrated ammonia R* and 9 volumes of *methanol R* and dilute to 100 ml with the same mixture of solvents.

Plate *TLC silica gel G plate R*.

Mobile phase *concentrated ammonia R*, *propanol R*, *alcohol R* (20:20:60 *V/V/V*).

Application 2 µl.

Development Over 3/4 of the plate.

Drying In air.

Detection Ultraviolet light at 365 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence 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 0.100 g of the substance to be examined in 5 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (a) Dissolve 0.100 g of *folic acid CRS* in 5 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b) Dissolve 20 mg of *pteroic acid R* in 5 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Mix 1.0 ml of this solution with 1.0 ml of reference solution (a) and dilute to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (d) Dissolve 10.0 mg of *N-(4-aminobenzoyl)*-L-*glutamic acid R* in 1 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (e) Dissolve 12.0 mg of *pteroic acid R* in 1 ml of a 28.6 g/l solution of *sodium carbonate R* and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

—*size*: l = 0.25 m, Ø = 4.0 mm,
—*stationary phase*: spherical *octylsilyl silica gel for chromatography R* (5 µm) with a carbon loading of 12.5 per cent, a specific surface of 350 m^2/g and a pore size of 10 nm.

Mobile phase Mix 12 volumes of *methanol R* and 88 volumes of a solution containing 11.16

g/l *potassium dihydrogen phosphate R* and 5.50 g/l of *dipotassium hydrogen phosphate R* solution.

Flow rate 0.6 ml/min.

Detection Spectrophotometer at 280 nm.

Injection 5 µl; inject the test solution and reference solutions (b), (c) (d) and (e).

Run time 3 times the retention time of folic acid.

Relative retention with reference to folic acid (retention time = about 8.5 min): impurity A = about 0.5; impurity B = about 0.6; impurity C = about 0.9; impurity E = about 1.27; impurity D = about 1.33; impurity F = about 2.2.

System suitability Reference solution (b):

— *resolution*: minimum 4.0 between the peaks due to folic acid and to impurity D.

Limits:

—*impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent),

—*impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.6 per cent),

—*any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

—*total of other impurities*: not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent),

—*disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Water *(2.5.12)*

5.0 per cent to 8.5 per cent, determined on 0.150 g.

Sulphated ash *(2.4.14)*

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography *(2.2.29)* as described in the test for related substances with the following modification.

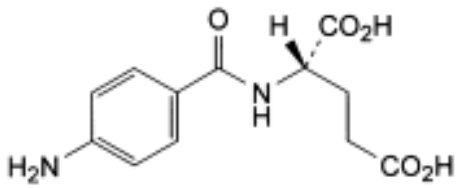
Injection Test solution and reference solution (a).

STORAGE

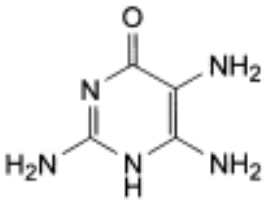
Protected from light.

IMPURITIES

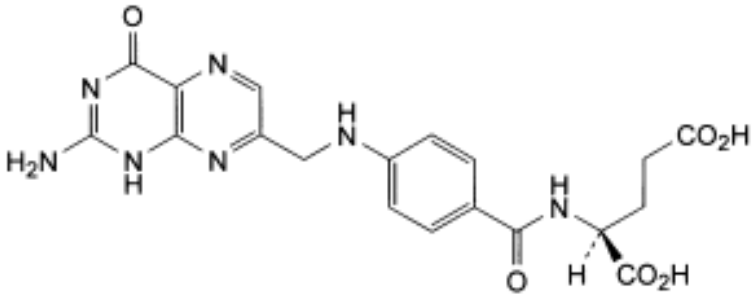
Specified impurities *A, B, C, D, E, F.*



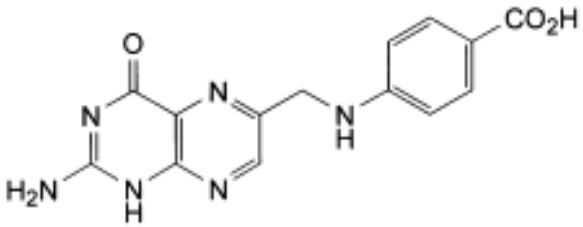
A. (2*S*)-2-[(4-aminobenzoyl)amino]pentanedioic acid (*N*-(4-aminobenzoyl)-L-glutamic acid),



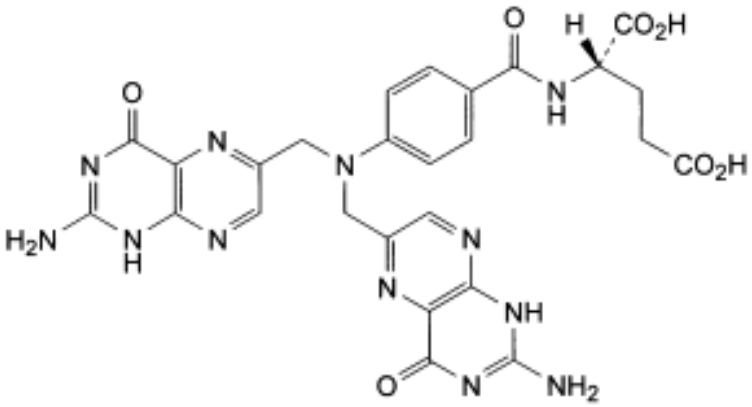
B. 2,5,6-triaminopyrimidin-4(1*H*)-one,



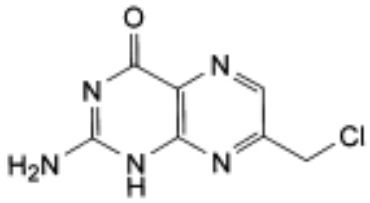
C. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-7-yl)methyl]amino]benzoyl]amino] pentanedioic acid (isofolic acid),



D. 4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoic acid (pteroic acid),



E. (2*S*)-2-[[4-[bis[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzoyl]amino] pentanedioic acid (6-pterinylfolic acid),



F. 2-amino-7-(chloromethyl)pteridin-4(1*H*)-one.

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