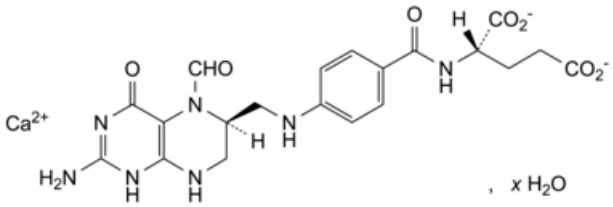
Edition: BP 2025 (Ph. Eur. 11.6 update)

Calcium Levofolinate Hydrate

General Notices

Calcium Levofolinate Pentahydrate

(Ph. Eur. monograph 1606)



$C_{20}H_{21}CaN_7O_7,xH_2O$ 511.5 (anhydrous substance)

Action and use

Antidote to folic acid antagonists.

Ph Eur

DEFINITION

Calcium (2*S*)-2-[4-[[[(6*S*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino]benzamido]pentanedioate hydrate.

Content

— *calcium levofolinate* ($C_{20}H_{21}CaN_7O_7$; M_r 511.5): 97.0 per cent to 102.0 per cent (anhydrous substance);

— *calcium* (Ca; A_r 40.08): 7.54 per cent to 8.14 per cent (anhydrous substance).

It contains a variable quantity of water.

CHARACTERS

Appearance

White or light yellow, amorphous or crystalline, hygroscopic powder.

Solubility

Slightly soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

It shows polymorphism (5.9).

IDENTIFICATION

First identification: A, B, D.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).
B. Infrared absorption spectrophotometry (2.2.24).

Comparison *calcium folinate CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in the minimum quantity of *water R* and add dropwise sufficient *acetone R* to produce a precipitate. Allow to stand for 15 min, collect the precipitate by centrifugation, wash the precipitate twice with a minimum quantity of *acetone R* and dry. Record new spectra using the residues.

C. Thin-layer chromatography (2.2.27).

Test solution Dissolve 15 mg of the substance to be examined in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 mL with the same solvent.

Reference solution Dissolve 15 mg of *calcium folinate CRS* in a 3 per cent *V/V* solution of *ammonia R* and dilute to 5 mL with the same solvent.

Plate *cellulose for chromatography F_{254} R* as the coating substance.

Mobile phase The lower layer of a mixture of 1 volume of *isoamyl alcohol R* and 10 volumes of a 50 g/L solution of *citric acid monohydrate R* previously adjusted to pH 8 with *ammonia R*.

Application 5 µL.

Development Over 2/3 of the plate.

Drying In 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.

D. It gives reaction (b) of calcium (2.3.1).

TESTS

Carry out the tests as rapidly as possible, protected from actinic light.

Solution S

Dissolve 0.40 g in *carbon dioxide-free water R*, heating at 40 °C if necessary, and dilute to 50.0 mL with the same solvent.

Appearance of solution

Solution S is clear (2.2.1).

pH (2.2.3)

7.5 to 8.5 for solution S.

Specific optical rotation (2.2.7)

-15.0 to -10.0 (anhydrous substance), measured at 25 °C.

Dissolve 0.200 g in *tris(hydroxymethyl)aminomethane solution R* previously adjusted to pH 8.1 with *sodium hydroxide solution R* or *hydrochloric acid R1* and dilute to 20.0 mL with the same solvent.

Absorbance (*2.2.25*)

Maximum 0.25, determined at 420 nm on solution S.

Ethanol

Head-space gas chromatography (*2.2.28*): use the standard additions method.

Test solution Dissolve 0.25 g of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dilute 0.750 g of *anhydrous ethanol R* to 1000.0 mL with *water R*.

Column:

— *material*: fused silica;

— *size*: *l* = 10 m, Ø = 0.32 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R*.

Carrier gas *nitrogen for chromatography R*.

Flow rate 4 mL/min.

Static head-space conditions that may be used:

— *equilibration temperature*: 80 °C;

— *equilibration time*: 20 min;

— *pressurisation time*: 30 s.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 14	80 → 220
Injection port		110
Detector		270

Detection Flame ionisation.

Injection At least 3 times.

Limit:

— *ethanol*: maximum 3.0 per cent.

Related substances

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

Test solution Dissolve 10.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *calcium folinate CRS* in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (c) Dissolve 5 mg of *calcium folinate for system suitability A CRS* (containing impurities A, B, C, D, E, F and I) in 5 mL of a 2.5 g/L solution of *sodium hydrogen carbonate R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Mix 165 mL of *methanol R* and 835 mL of a solution containing 4.0 mL of *tetrabutylammonium dihydrogen phosphate solution R* and 1.42 g of *disodium hydrogen phosphate dihydrate R* in *water for chromatography R*, previously adjusted to pH 7.7 with *phosphoric acid R* or *dilute sodium hydroxide solution R*.

Flow rate 1.25 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 10 µL of the test solution and reference solutions (b) and (c).

Run time 4 times the retention time of folinic acid.

Identification of impurities Use the chromatogram supplied with *calcium folinate for system suitability A CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, D, E, F and I.

Relative retention With reference to folinic acid (retention time = about 12.0 min): impurity E = about 0.4; impurity A = about 0.6; impurity F = about 0.7; impurity B = about 0.8; impurity I = about 1.3 (may be eluted as 1 or 2 peaks); impurity D = about 2.1; impurity C = about 2.6.

System suitability Reference solution (c):

— *resolution*: minimum 2.0 between the peaks due to impurities A and F.

Calculation of percentage contents:

— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.6; impurity B = 0.5; impurity C = 0.6; impurity D = 0.3; impurity E = 0.6; impurity F = 0.6; impurity I = 0.6;

— for each impurity, use the concentration of calcium levofolinate hydrate in reference solution (b).

Limits:

— *impurities A, E*: for each impurity, maximum 0.3 per cent;

— *impurities B, C, D, F*: for each impurity, maximum 0.2 per cent;

— *impurity I*: maximum 0.2 per cent, for the sum of the areas of the 2 peaks;

— *unspecified impurities*: for each impurity, maximum 0.20 per cent;

— *total*: maximum 1.5 per cent;

— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Impurity H

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dissolve 10.0 mg of *calcium folinate CRS* in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 100.0 mL with *water R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4 mm;

— *stationary phase*: *human albumin coated silica gel for chiral separation R* (5 µm);

— *temperature*: 40 °C.

Mobile phase Dissolve 9.72 g of *sodium dihydrogen phosphate R* in 890 mL of *water for chromatography R* and adjust to pH 5.0 with *sodium hydroxide solution R*; add 100 mL of *2-propanol R* and 10 mL of *acetonitrile R*.

Flow rate 1 mL/min.

Detection Spectrophotometer at 286 nm.

Injection 10 µL.

Retention times Levofolinic acid = about 9 min; impurity H = about 19 min.

System suitability:

— *resolution*: minimum 5.0 between the peaks due to levofolinic acid and impurity H in the chromatogram obtained with reference solution (a). The sum of the areas of the 2 peaks is 100 per cent. The peak area of impurity H is 48 per cent to 52 per cent. In the chromatogram obtained with reference solution (b) 2 clearly visible peaks are obtained.

Limit:

— *impurity H*: maximum 0.5 per cent.

Chlorides

Maximum 0.5 per cent.

Dissolve 0.300 g in 50 mL of *water R* heating at 40 °C if necessary. Add 10 mL of *dilute nitric acid R* and titrate with *0.005 M silver nitrate* determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.005 M silver nitrate* is equivalent to 0.177 mg of Cl.

Water

(*2.5.12*): 10.0 per cent to 17.0 per cent.

Dissolve 0.100 g in a mixture of 15 mL of *formamide R* and 50 mL of the titration solvent. Stir for about 6 min before titrating and use a suitable titrant that does not contain pyridine.

ASSAY

Carry out the assays as rapidly as possible, protected from actinic light.

Calcium

Dissolve 0.400 g in 150 mL of *water R* and dilute to 300 mL with the same solvent. Carry out the complexometric titration of calcium (*2.5.11*).

1 mL of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

Calcium folinate

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

Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{20}H_{21}CaN_7O_7$ taking into account the assigned content of *calcium folinate CRS*.

STORAGE

In an airtight container, protected from light. If the substance is sterile, the container is also sterile and tamper-evident.

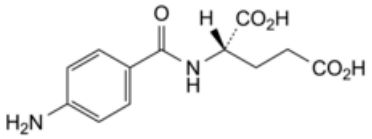
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

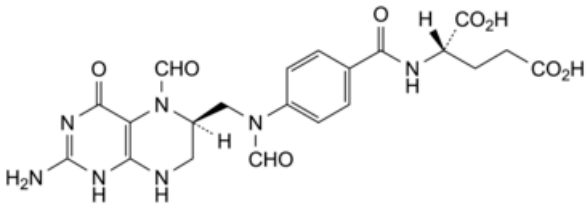
IMPURITIES

Specified impurities A, B, C, D, E, F, H, I.

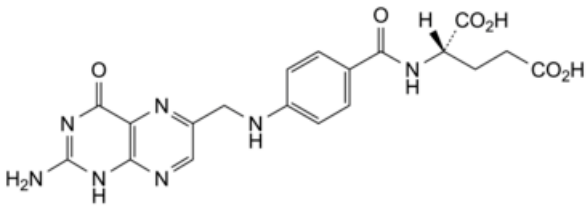
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for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



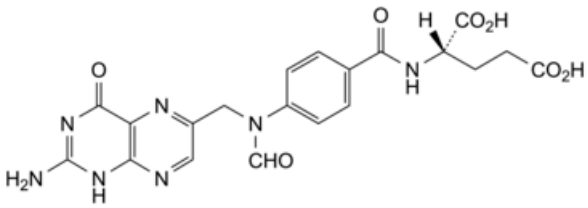
A. (2*S*)-2-(4-aminobenzamido)pentanedioic acid,



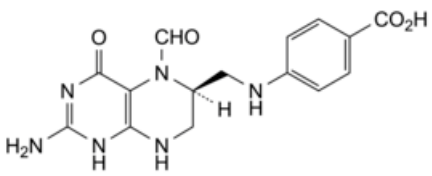
B. (2*S*)-2-[4-[[[(6*R*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl](formyl)amino]benzamido]pentanedioic acid (5,10-diformyltetrahydrolevofolic acid),



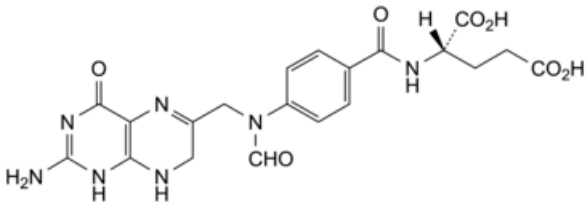
C. (2*S*)-2-[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzamido]pentanedioic acid (folic acid),



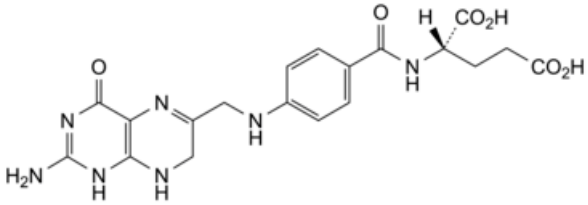
D. (2*S*)-2-[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl](formyl)amino]benzamido]pentanedioic acid (10-formylfolic acid),



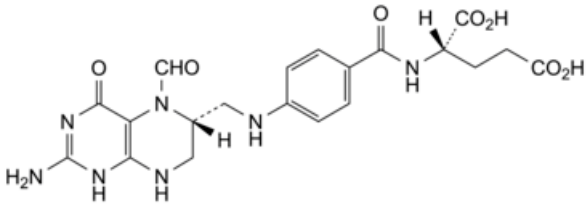
E. 4-[[[(6*S*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino]benzoic acid ((6*S*)-5-formyltetrahydropteroic acid),



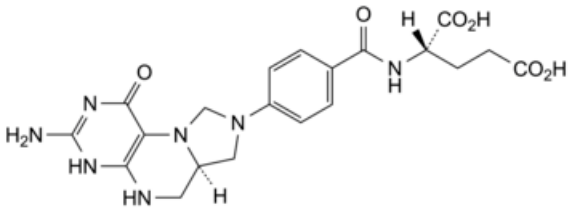
F. (2*S*)-2-[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl](formyl)amino]benzamido]pentanedioic acid (10-formyldihydrofolic acid),



G. (2*S*)-2-[4-[[(2-amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino]benzamido]pentanedioic acid (dihydrofolic acid),



H. (2*S*)-2-[4-[[[(6*R*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl]amino]benzamido]pentanedioic acid (dextrofolinic acid),



I. (2*S*)-2-[4-[(6a*R*)-3-amino-1-oxo-1,4,5,6,6a,7-hexahydroimidazo[1,5-*f*]pteridin-8(9*H*)-yl]benzamido]pentanedioic acid ((6a*R*)-5,10-methylenetetrahydrofolic acid).

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