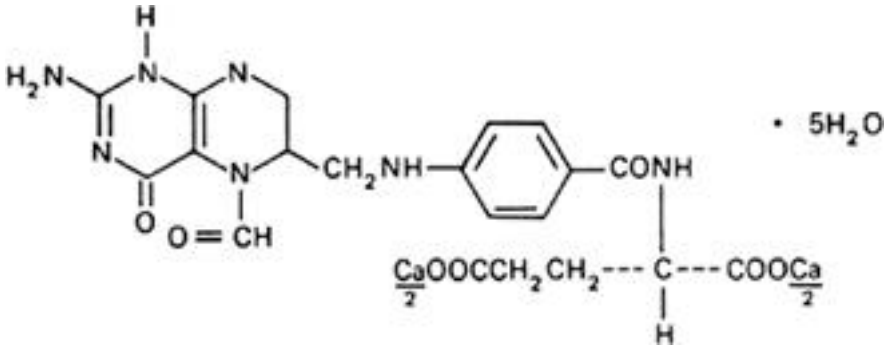
Calcium folinate (Calcii folinas)

Molecular formula. $C_{20}H_{21}CaN_7O_7,5H_2O$

Relative molecular mass. 601.6

Graphic formula.



Chemical name.

Calcium *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-L-glutamate (1:1) pentahydrate; calcium *N*-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-L-glutamate (1:1) pentahydrate; CAS Reg. No. 6035-45-6 (pentahydrate).

Other name. Leucovorin calcium.

Description. A white or creamy white powder; odourless.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Cytotoxic drug.

Storage. Calcium folinate should be kept in a well-closed container, protected from light.

Additional information. CAUTION: Calcium folinate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Calcium folinate contains not less than 95.0% and not more than 105.0% of $C_{20}H_{21}CaN_7O_7$, calculated with reference to the anhydrous substance.

Identity tests

• Either tests A and C or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from calcium folinate RS or with the *reference spectrum* of calcium folinate.

B. Dissolve 20 mg in 3.0 mL of water, add 0.5 mL of hydrochloric acid (~70 g/l) TS and 0.5 mL of sodium nitrite (100 g/l) TS. Shake for 2 minutes and add 1.5 mL of 2-naphthol TS1; a yellow-brown precipitate appears and the solution turns green.

C. Dissolve 20 mg in 2.0 mL of water and add 1.0 mL of ammonium oxalate (25 g/l) TS; a white precipitate is produced, which is insoluble in acetic acid (~300 g/l) TS and ammonia (~260 g/l) TS, but is soluble in hydrochloric acid (~70 g/l) TS.

D. Dissolve 20 mg in 5 mL of water and add 1.0 mL of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Add a few drops of nitric acid (~130 g/l) TS; the precipitate dissolves.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not less than 0.080 g/g and not more than 0.150 g/g.

Assay

• Use freshly deionized water throughout the procedure, and perform the assay in low-actinic glassware or protect the solutions containing calcium folinate from light. Complete the assay without prolonged interruption.

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a column 30 cm long and 4 mm in internal diameter, packed with particles of porous silica gel or ceramic, 5-10 µm in diameter, the surface of which has been modified with chemically bonded octadecylsilyl groups.

As the mobile phase, use a mixture of 15 mL of tetrabutylammonium hydroxide/methanol TS with 835 mL of water, add 125 mL of acetonitrile R, adjust the pH to 7.5 ± 0.1 with sodium dihydrogen phosphate (275 g/l) TS, dilute with water to 1000 mL, and filter. Adjust the concentration of acetonitrile, if necessary.

Dilute the following solution for use in the preparation of the test solutions: To 15 mL of tetrabutylammonium hydroxide/methanol TS add 900 mL of water, adjust the pH to 7.5 ± 0.1 with sodium dihydrogen phosphate (275 g/l) TS, dilute with water to 1000 mL, and mix. Weigh accurately a quantity of calcium folinate RS, dissolve it in the above solution and dilute with the same solution to contain about 175 µg per mL (solution A). Dissolve 20 mg of the substance to be examined in a sufficient volume of the above solution to produce 100 mL, and mix (solution B). For the system suitability test, dissolve a quantity of folic acid RS in the above solution and dilute with the same solution to contain about 175 µg per mL. Mix 1 part of this solution with 4 parts of solution A (solution C).

Operate at a flow rate of 1-2 mL per minute. As detector use an ultraviolet spectrophotometer at a wavelength of about 254 nm, fitted with a suitable recorder.

Make 6 replicate injections, each of 15 µl of solution C. The resolution factor between calcium folinate and folic acid should be not less than 3.6, with a relative standard deviation for the calcium folinate peak of not more than 2.0%. The relative retention times for calcium folinate and folic acid are 1.0 and about 1.6, respectively.

Then inject 15 µl of each of solutions A and B. Measure the peak responses at the corresponding retention times and calculate the quantity, in %, of $C_{20}H_{21}CaN_7O_7$, using the following formula: $100(0.1C)(r_U/r_S)$ in which C is the concentration in µg per mL of calcium folinate RS in solution A, and r_U and r_S are the peak responses obtained from solutions B and A, respectively.

Additional requirements for Calcium folinate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.5 IU of endotoxin RS per mg.