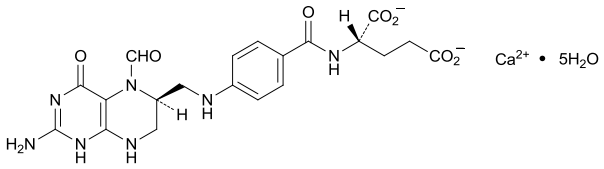
# Calcium Levofolinate Hydrate

レボホリナートカルシウム水和物



$C_{20}H_{21}CaN_7O_7.5H_2O$: 601.58

Monocalcium *N*-[4-({[(6*S*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl}amino)benzoyl]-L-glutamate pentahydrate

[*419573-16-3*]

Calcium Levofolinate Hydrate contains not less than 97.0% and not more than 102.0% of calcium levofolinate ($C_{20}H_{21}CaN_7O_7$: 511.50), calculated on the anhydrous and residual solvent-free basis.

**Description** Calcium Levofolinate Hydrate occurs as a white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

Optical rotation $[\alpha]_D^{25}$: −10 – −15° (0.25 g calculated on the anhydrous and residual solvent-free basis, 0.2 mol/L tris buffer solution (pH 8.1), 25 mL, 100 mm).

**Identification** **(1)** Determine the absorption spectrum of a solution of Calcium Levofolinate Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Calcium Levofolinate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Calcium Levofolinate Hydrate (1 in 200) responds to the Qualitative Tests *<1.09>* (2) and (3) for calcium salt.

**pH** *<2.54>* To 0.4 g of Calcium Levofolinate Hydrate add 50 mL of freshly boiled and cooled water, and warm to 40℃, if necessary, to dissolve: 7.5 to 8.5.

**Purity** **(1)** Clarity and color of solution－To 0.4 g of Calcium Levofolinate Hydrate add 50 mL of water, and warm to 40℃, if necessary, to dissolve: the solution is clear, and the absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry *<2.24>* is not more than 0.25.

**(2)** Chloride－To 0.300 g of Calcium Levofolinate Hydrate add 50 mL of water, warm to 40℃, if necessary, to dissolve, add 10 mL of 2 mol/L nitric acid TS, and titrate *<2.50>* with 0.005 mol/L silver nitrate VS (potentiometric titration) (not more than 0.5%).

Each mL of 0.005 mol/L silver nitrate VS = 0.177 mg of Cl

**(3)** Heavy metals *<1.07>*－Proceed with 1.0 g of Calcium Levofolinate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(4)** Platinum Being specified separately when the drug is granted approval based on the Law (not more than 5 ppm).

**(5)** Related substances－Dissolve 20 mg of Calcium Levofolinate Hydrate in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than levofolinate from the sample solution is not larger than the peak area of levofolinate from the standard solution, and the total area of the peaks other than levofolinate from the sample solution is not larger than 5 times the peak area of levofolinate from the standard solution.

*Operating conditions*－

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of levofolinate, beginning after the solvent peak.

*System suitability*－

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 25 mL. Confirm that the peak area of levofolinate obtained with 20 μL of this solution is equivalent to 14 to 26% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of levofolinate are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of levofolinate is not more than 2.0%.

**(6)** Diastereomer－Dissolve 50 mg of Calcium Levofolinate Hydrate in 100 mL of water, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak of diastereomer, having a relative retention time of about 2.0 to levofolinate, is not more than 0.3%.

*Operating conditions* －

Detector: An ultraviolet absorption photometer (wavelength: 286 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography covalently binding with human albumin (5 μm in particle diameter).

Column temperature: A constant temperature of about 40℃.

Mobile phase: Dissolve 3.4 g of sodium dihydrogen phosphate dihydrate in 870 mL of water, adjust to pH 4.9 with sodium hydroxide TS or phosphoric acid, and add 110 mL of 2-propanol and 20 mL of acetonitrile.

Flow rate: Adjust so that the retention time of levofolinate is about 16 minutes.

*System suitability* －

Test for required detectability: Dissolve 20 mg of Calcium Folinate RS in water to make 100 mL. To 1 mL of this solution add the sample solution to make 20 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of diastereomer obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, levofolinate and the diastereomer are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of diastereomer is not more than 2.0%.

**Water** <*2.48*> 12.0 – 17.0% (0.2 g, volumetric titration, direct titration ).

**Assay** Weigh accurately about 10 mg each of Calcium Levofolinate Hydrate and Calcium Folinate RS (separately determine the water <*2.48*> in the same manner as Calcium Folinate), each separately in water to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area, $A_T$, of levofolinate with the sample solution, and the peak area, $A_S$, of folinate with the standard solution.

Amount (mg) of calcium levofolinate ($C_{20}H_{21}CaN_7O_7$)
$= M_S \times A_T / A_S$

$M_S$: Amount (mg) of Calcium Folinate RS taken, calculated on the anhydrous basis

*Operating conditions* －

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45℃.

Mobile phase: Adjust the pH of a mixture of diluted 0.05 mol/L disodium hydrogen phosphate TS (4 in 25), methanol and tetrabutylammonium hydroxide TS (385:110:4) to 7.5 with phosphoric acid.

Flow rate: Adjust so that the retention time of folinate is about 10 minutes.

*System suitability* －

System performance: Dissolve 10 mg of folic acid in 50 mL of the mobile phase. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, folinate and folic acid are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of folinate is not more than 1.0%.

**Containers and storage** Containers－Tight containers.

Storage－Light-resistant.

***Add the following to 9.41 Reagents, Test Solutions:***

**0.2 mol/L Tris buffer solution, pH 8.1** Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL, and adjust to pH 8.1 with hydrochloric acid.