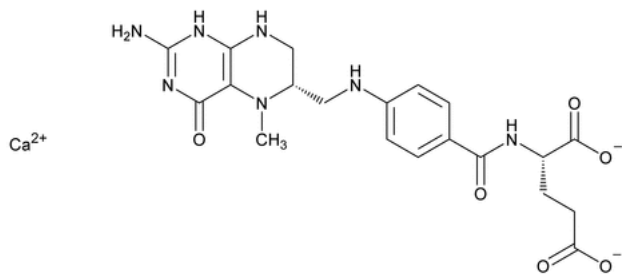


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Calcium L-5-Methyltetrahydrofolate



$C_{20}H_{23}CaN_7O_6 \cdot xH_2O$ $C_{20}H_{23}CaN_7O_6$ (anhydrous) 497.52
N-[4-[[[(2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-(6S)-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, calcium salt (1:1);
N-{4-[[[(6S)-2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl]amino]-benzoyl]-L-glutamic acid, calcium salt (1:1) CAS RN®:
151533-22-1.

DEFINITION
Calcium L-5-Methyltetrahydrofolate contains NLT 95.0% and NMT 102.0% of calcium 5-methyltetrahydrofolate ($C_{20}H_{23}CaN_7O_6$), the sum of the L- and D-diastereoisomers, calculated on the anhydrous and solvent-free basis, of which NMT 1.0% corresponds to calcium D-5-methyltetrahydrofolate.

IDENTIFICATION

Change to read:

- **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy: 197K**

[NOTE—If the spectra obtained show differences, dissolve the substance to be examined and the [USP Calcium D,L-5-Methyltetrahydrofolate RS](#) (ERR 1-Nov-2022) separately in the minimum quantity of water, and add dropwise sufficient acetone to produce a precipitate. Allow to stand for 15 min, centrifuge to collect the precipitate, wash the precipitate twice with a minimum quantity of acetone, and dry. Record new spectra using the residues.]

- **B. IDENTIFICATION TESTS—GENERAL, Calcium (191):** A 5-mg/mL solution meets the requirements.
- **C. HPLC:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. It complies with the acceptance criteria of the test for *Enantiomeric Purity*.

ASSAY

Change to read:

- **PROCEDURE**

Buffer: 7.8 g/L of sodium dihydrogen phosphate dihydrate in water
Solution A: Adjust the *Buffer* with 32% (w/v) sodium hydroxide solution to a pH of 6.5.
Solution B: Methanol and *Buffer* (35:65). Adjust with 32% (w/v) sodium hydroxide solution to a pH of 8.0.
Mobile phase: Gradient elution. See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
14	45	55
17	0	100
24	0	100
24.01	100	0

Time (min)	Solution A (%)	Solution B (%)
33	100	0

[NOTE—After analysis the column should be flushed and stored in a mixture of methanol and water (85:15).]

System suitability solution: Transfer 25 mg of [USP Folic Acid RS](#) and 25 mg of [USP 4-Aminobenzoylglutamic Acid RS](#) to a 100-mL volumetric flask. Add about 15 mg each of sodium hydrogen carbonate and sodium carbonate to the flask, add sufficient water, sonicate to dissolve, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask containing 50 mg of [▲USP Calcium D,L-5-Methyltetrahydrofolate RS](#)▲ (ERR 1-Nov-2022), dissolve, and dilute with water to volume.

[NOTE—The following Standard and Sample solutions must be injected immediately after preparation and injected only once.]

Standard solution: 0.5 mg/mL of [▲USP Calcium D,L-5-Methyltetrahydrofolate RS](#)▲ (ERR 1-Nov-2022) in water

Sample solution: 0.5 mg/mL of Calcium L-5-Methyltetrahydrofolate in water

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 32°

Flow rate: 1.1 mL/min

Injection volume: 10 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the *System suitability solution* the relative retention times of the component peaks are listed in [Table 2](#). The L- and D-isomers of 5-methyltetrahydrofolate co-elute as a single peak. The 4a-hydroxy-5-methyltetrahydrofolic acid, 5-methyltetrahydropteroic acid, and dimethyltetrahydrofolic acid are included as minor components in [▲USP Calcium D,L-5-Methyltetrahydrofolate RS](#)▲ (ERR 1-Nov-2022).]

Suitability requirements

Resolution: *System suitability solution*

NLT 6 between 4-aminobenzoylglutamic acid and 4a-hydroxy-5-methyltetrahydrofolic acid

NLT 8 between folic acid and 5-methyltetrahydrofolic acid

NLT 15 between 5-methyltetrahydrofolic acid and dimethyltetrahydrofolic acid

Relative standard deviation: Prepare three separate *Standard solutions*, and inject each immediately and only one time. NMT 2.0%; peak response factor from three injections

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of calcium 5-methyltetrahydrofolate ($C_{20}H_{23}CaN_7O_6$), the sum of the L- and D-diastereoisomers, in the portion of Calcium L-5-Methyltetrahydrofolate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of [▲USP Calcium D,L-5-Methyltetrahydrofolate RS](#)▲ (ERR 1-Nov-2022) in the *Standard solution* (mg/mL)

C_U = concentration of Calcium L-5-Methyltetrahydrofolate in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• CHLORIDE

Sample: 300 mg

Blank: Mix 1 mL of nitric acid with 75 mL of water.

Titrimetric system

(See [Titrimetry \(541\)](#).)

Mode: Direct titration

Titrant: 0.005 M silver nitrate VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 75 mL of water (heat to maximum of 40°), add 1 mL of nitric acid, and titrate with the *Titrant*. Perform a *Blank* determination, and make any necessary correction.

Calculate the percentage of chloride (Cl) in the *Sample* taken: