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Calcium L-5-Methyltetrahydrofolate Capsules

DEFINITION

Calcium L-5-Methyltetrahydrofolate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium L-5-methyltetrahydrofolate ($C_{20}H_{23}CaN_7O_6$).

IDENTIFICATION

• **A. HPLC:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Strength*, and to the L-isomer of the *Standard solution* in the test for *Enantiomeric Purity*.

STRENGTH

• **PROCEDURE**

- Antioxidant solution:** 1.5% [sodium sulfite](#) in water
- 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
33	100	0

System suitability solution: Transfer 25 mg of [USP Folic 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](#), dissolve, and dilute with water to volume.

Standard solution: 0.1 mg/mL of [USP Calcium D,L-5-Methyltetrahydrofolate RS](#) in *Antioxidant solution*

Sample solution: Remove, as completely as possible, the contents of NLT 30 Capsules, and weigh accurately. Transfer a portion of the Capsule contents, nominally equivalent to 2.5 mg of calcium L-5-methyltetrahydrofolate, to a 25-mL volumetric flask. Add 20 mL of *Antioxidant solution* and sonicate for 20 min with occasional shaking, cool to room temperature, dilute with *Antioxidant solution* to volume, mix well, and filter.

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 folic acid and L- and D-isomers of 5-methyltetrahydrofolate, which co-elute as a single peak, are 0.85 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 8 between folic acid and 5-methyltetrahydrofolic acid, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium L-5-methyltetrahydrofolate ($C_{20}H_{23}CaN_7O_6$) in the portion of Capsules 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](#) in the *Standard solution* (mg/mL)

C_U = nominal concentration of calcium L-5-methyltetrahydrofolate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• ENANTIOMERIC PURITY

Buffer: 4.54 g/L of sodium dihydrogen phosphate dihydrate in water

Mobile phase: Acetonitrile and *Buffer* (3:97). Adjust with 32% (w/v) sodium hydroxide to a pH of 6.8.

Standard solution: 0.4 mg/mL of [USP Calcium D,L-5-Methyltetrahydrofolate RS](#) in water

Sample solution: Filtered portion, equivalent to 0.4 mg/mL of calcium L-5-methyltetrahydrofolate, from the contents of NLT 30 Capsules, in water

System suitability solution: Transfer 0.2 mL of *Standard solution* to a 10-mL volumetric flask, and dilute with *Sample solution* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.0-mm × 15-cm; 5-μm packing L79¹

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 10 μL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times of L-5-methyltetrahydrofolate and D-5-methyltetrahydrofolate are about 1 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 1.5 between L-5-methyltetrahydrofolate and D-5-methyltetrahydrofolate

Analysis

Sample: *Sample solution*

Calculate the percentage of D-5-methyltetrahydrofolate in the portion of calcium L-5-methyltetrahydrofolate taken:

$$\text{Result} = [r_D/(r_D + r_L)] \times 100$$

r_D = peak response of D-5-methyltetrahydrofolate from the *Sample solution*

r_L = peak response of L-5-methyltetrahydrofolate from the *Sample solution*

Acceptance criteria: NMT 1.0%

PERFORMANCE TESTS

- [DISINTEGRATION AND DISSOLUTION \(2040\)](#), [Disintegration](#): Meet the requirements
- [WEIGHT VARIATION OF DIETARY SUPPLEMENTS \(2091\)](#): Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store in a tight, light-resistant container, in a cool and dry place.
- [USP REFERENCE STANDARDS \(11\)](#)
[USP Calcium D,L-5-Methyltetrahydrofolate RS](#)
[USP Folic Acid RS](#)