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Leucovorin Calcium Tablets

DEFINITION
Leucovorin Calcium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

IDENTIFICATION

- **A.**

Sample: Equivalent to 200 mg of leucovorin calcium from finely powdered Tablets

Analysis: Transfer the *Sample* to a conical flask. Add 10 mL of water, shake vigorously, sonicate for 10 min, and filter. Transfer the filtrate to a stoppered centrifuge tube, add 125 mg of ammonium oxalate, shake vigorously, and centrifuge until a clear supernatant is obtained. Transfer the supernatant to another stoppered centrifuge tube, add 1 mL of methanol and 3 drops of hydrochloric acid, and shake vigorously. If the preparation is cloudy, add methanol until a clear solution is obtained, and filter if necessary to remove any undissolved material. Cool the preparation at 0° until a precipitate forms, and centrifuge for 1–2 min. [NOTE—The cooling and centrifuging steps may be repeated if necessary to increase the amount of precipitate collected.] Decant the supernatant, add 2 mL of methanol to the tube, shake vigorously to dissolve the precipitate, and transfer the contents to a beaker. Evaporate under a current of air to dryness, and dry the residue at 50° for 30 min.

Acceptance criteria: The IR absorption spectrum of a potassium bromide dispersion of the residue exhibits maxima only at the same wavelengths as that of a similar preparation of USP Leucovorin Calcium RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Diluent: Methanol and water (20:80)

Mobile phase: 5 mM tetrabutylammonium phosphate in *Diluent*. Adjust with 50% (w/v) sodium hydroxide to a pH of 7.5.

Standard solution: 0.5 mg/mL of USP Leucovorin Calcium RS and 10 µg/mL of USP 10-Formylfolic Acid RS in water

Sample solution: Transfer finely powdered Tablets (NLT 20), equivalent to 50 mg of leucovorin, to a 100-mL volumetric flask. Add 50 mL of water, sonicate for 30 min, dilute with water to volume, mix, and filter.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 2.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for leucovorin and 10-formylfolic acid are about 1.0 and 2.3, respectively.]

Suitability requirements

Resolution: NLT 1.5 between leucovorin and 10-formylfolic acid

Relative standard deviation: NMT 2.0% for leucovorin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of the *Sample solution*

r_S = peak area of the *Standard solution*

C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of leucovorin in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of leucovorin, 473.45

M_{r2} = molecular weight of leucovorin calcium, 511.50

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Detector: UV, at a maximum of about 284 nm

Standard solution: USP Leucovorin Calcium RS in *Medium*

Sample solution: Use filtered portion of solution under test, and dilute with water if necessary to a concentration similar to that of the *Standard solution*.

Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

D = dilution factor

M_{r1} = molecular weight of leucovorin, 473.45

M_{r2} = molecular weight of leucovorin calcium, 511.50

L = label claim (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) is dissolved.

Change to read:

• **Uniformity of Dosage Units** ⟨905⟩**:** ▲Meet the requirements▲ (CN 1-Aug-2023)

Analysis for content uniformity

Standard solution: 10 µg/mL of USP Leucovorin Calcium RS

Sample solution: 10 µg/mL of leucovorin calcium, use individual intact Tablets.

Blank: Water

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Cell: 1 cm

Analytical wavelength: UV, at maxima about 284 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$) in each Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

C_S = concentration of USP Leucovorin Calcium RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of leucovorin in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of leucovorin, 473.45

M_{r2} = molecular weight of leucovorin calcium, 511.50

▲▲ (CN 1-Aug-2023)

IMPURITIES

• **Organic Impurities**

Diluent, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = response of each impurity peak

r_T = sum of the responses of all the peaks

Acceptance criteria

Individual impurities: NMT 2.5%

Total impurities: NMT 4.0 %

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, protected from light, at controlled room temperature.

• **USP Reference Standards** ⟨11⟩

USP 10-Formylfolic Acid RS

USP Leucovorin Calcium RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
LEUCOVORIN CALCIUM TABLETS	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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