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Leucovorin Calcium Injection

DEFINITION

Leucovorin Calcium Injection is a sterile solution of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$) in Water for Injection. It contains NLT 90.0% and NMT 120.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

IDENTIFICATION

Change to read:

- A. **▲ SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy: 197K ▲** (CN 1-MAY-2020)

Analysis: Transfer a volume of Injection, equivalent to 6 mg of leucovorin calcium, to a glass-stoppered, 50-mL centrifuge tube. Add 40 mL of acetone, mix, centrifuge for a few min, and decant and discard the liquid phase. Repeat the washing process with an additional 40 mL of acetone. Dry the precipitate so obtained with a stream of dry nitrogen.

Acceptance criteria: Meets the requirements

ASSAY

• PROCEDURE

Use only freshly deionized water wherever water is specified throughout this *Procedure*. Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light. Complete the assay without prolonged interruption.

Solution A: 250 mg/mL of tetrabutylammonium hydroxide in methanol

Solution B: 276 mg/mL of monobasic sodium phosphate monohydrate in water

Mobile phase: Mix 15 mL of *Solution A* with 835 mL of water. Add 125 mL of acetonitrile, adjust with *Solution B* to an apparent pH of 7.5 ± 0.1 , dilute with water to 1000 mL, and filter. Adjust the concentration of acetonitrile, if necessary.

Diluent: Mix 15 mL of *Solution A* with 900 mL of water, and adjust with *Solution B* to a pH of 7.5 ± 0.1 . Dilute with water to 1000 mL.

Standard solution: 0.175 mg/mL of [USP Leucovorin Calcium RS](#) in *Diluent*

Sample solution: Transfer a measured volume of Injection, equivalent to 9 mg of leucovorin, to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pipet 25 mL of this solution into a 60-mL separator, add 25 mL of methylene chloride, shake the mixture, allow the layers to separate, and discard the methylene chloride extract. Repeat the extraction with two more 25-mL portions of methylene chloride, discarding the methylene chloride extracts. Filter the aqueous layer, discarding the first 5 mL of the filtrate, and collect the remaining filtrate in a glass-stoppered conical flask.

System suitability stock solution: 0.175 mg/mL of folic acid in *Diluent*

System suitability solution: *System suitability stock solution* and *Standard solution* (1:4)

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1–2 mL/min

Injection volume: 15 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for leucovorin and folic acid are 1.0 and about 1.6, respectively.]

Suitability requirements

Resolution: NLT 3.6 between leucovorin calcium and folic acid

Relative standard deviation: NMT 2.0%

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 Injection taken:

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

r_U = peak response from the *Sample solution*

- r_s = peak response from 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%–120.0%

SPECIFIC TESTS

- **pH (791):** 6.5–8.5
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 1.95 USP Endotoxin Units/mg of leucovorin calcium
- **OTHER REQUIREMENTS:** It meets the requirements in [Injections and Implanted Drug Products \(1\)](#).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose, light-resistant containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS (11).**
[USP Leucovorin Calcium RS](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
LEUCOVORIN CALCIUM INJECTION	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:
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