

Status: Currently Official on 18-Feb-2025  
Official Date: Official as of 01-May-2020  
Document Type: USP Monographs  
DocId: GUID-7605F6B2-4F10-49B8-8B9C-49536D6F3D64\_4\_en-US  
DOI: [https://doi.org/10.31003/USPNF\\_M44580\\_04\\_01](https://doi.org/10.31003/USPNF_M44580_04_01)  
DOI Ref: 58h87

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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  $7.5 \pm 0.1$ , 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  $7.5 \pm 0.1$ . 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  $\times$  30-cm; packing L1

**Flow rate:** 1–2 mL/min

**Injection volume:** 15  $\mu$ L

**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	<a href="#">Documentary Standards Support</a>	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services <a href="mailto:RSTECH@usp.org">RSTECH@usp.org</a>	SM32020 Small Molecules 3

**Chromatographic Database Information:** [Chromatographic Database](#)**Most Recently Appeared In:**

Pharmacopeial Forum: Volume No. Information currently unavailable

**Current DocID: GUID-7605F6B2-4F10-49B8-8B9C-49536D6F3D64\_4\_en-US****DOI:** [https://doi.org/10.31003/USPNF\\_M44580\\_04\\_01](https://doi.org/10.31003/USPNF_M44580_04_01)**DOI ref:** [58h87](#)