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# Leucovorin Calcium for Injection

## DEFINITION

Leucovorin Calcium for Injection contains an amount of leucovorin calcium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of leucovorin ($C_{20}H_{23}N_7O_7$).

## IDENTIFICATION

***Change to read:***

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

**Standard solution:** 0.0215 mg/mL of USP Leucovorin Calcium RS in water

**Sample solution:** Nominally 0.02 mg/mL of leucovorin prepared by constituting Leucovorin Calcium for Injection in water

**Acceptance criteria:** Meets the requirements

• **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

***Change to read:***

• **Procedure**

Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light.

**Solution A:** Dissolve 2.6 mL of tetrabutylammonium hydroxide solution (40% in water) and 2.8 g of disodium hydrogen phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.8.

**Mobile phase:** Methanol and *Solution A* (150:850)

**Standard solution:** 0.12 mg/mL of USP Leucovorin Calcium RS in water. Sonicate as necessary to dissolve.

**Sample solution:** Nominally 0.1 mg/mL of leucovorin from NLT 4 vials 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

**Temperatures**

**Autosampler:** 10°

**Column:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

▲▲ (ERR 1-May-2020)

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**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 Leucovorin Calcium for Injection taken:

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

$r_U$ = peak response of leucovorin from the *Sample solution*

$r_S$ = peak response of leucovorin 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%–110.0%

## PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩:** Meets the requirements

## IMPURITIES

• **Organic Impurities**

Use low-actinic glassware for solutions containing leucovorin calcium, and otherwise protect the solutions from unnecessary exposure to light.

**Solution A, Mobile phase, Standard solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 1.2 mg/mL of USP Leucovorin Calcium RS and 12 µg/mL each of 7,8-dihydrofolic acid and USP Folic Acid RS in water. Sonicate as necessary to dissolve.

**Sensitivity solution:** 0.4 µg/mL of USP Leucovorin Calcium RS in water

**Sample solution:** Nominally 1 mg/mL of leucovorin from NLT 4 vials in water

**System suitability**

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

**Suitability requirements**

**Resolution:** NLT 1.5 between leucovorin and 7,8-dihydrofolic acid, *System suitability solution*

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

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Leucovorin Calcium for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

$r_U$ = peak response of each impurity from the *Sample solution*

$r_S$ = peak response of leucovorin 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

$F$ = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*. Disregard any impurity peaks less than 0.03%.

**Table 1**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| 5-Formyl-5,6,7,8-tetrahydropteroic acid[a] | 0.51 | 1.0 | 2.0 |
| 4-Aminobenzoylglutamic acid[b] | 0.59 | 1.0 | 2.0 |
| 10-Formyldihydrofolic acid[c] | 0.76 | 0.47 | 2.0 |
| 5,10-Diformyltetrahydrofolic acid[d] | 0.85 | 0.49 | 2.0 |
| Leucovorin | 1.0 | 1.0 | — |
| 7,8-Dihydrofolic acid[e] | 1.15 | 1.0 | 2.0 |

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| 10-Formylfolic acid[f] | 2.11 | 0.60 | 2.0 |
| Folic acid | 2.50[g] | 1.0 | 2.0 |
| Individual, unspecified impurity | — | 1.0 | 0.5 |
| Total impurities | — | — | 2.5 |

[a] 4-{[(2-Amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]amino}benzoic acid.

[b] *N*-(4-Aminobenzoyl)-L-glutamic acid.

[c] (4-{*N*-[(2-Amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]formamido}benzoyl)-L-glutamic acid.

[d] (4-{*N*-[(2-Amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]formamido}benzoyl)-L-glutamic acid.

[e] *N*-(4-{[(2-Amino-4-oxo-1,4,7,8-tetrahydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamic acid.

[f] (4-{*N*-[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]formamido}benzoyl)-L-glutamic acid.

[g] Relative retention time may vary from 2.6 to 3.1 and may be compared with the result obtained from the *System suitability solution*.

## SPECIFIC TESTS

- **pH ⟨791⟩:** 6.5–8.5
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections
- **Sterility Tests ⟨71⟩:** Meets the requirements
- **Bacterial Endotoxins Test ⟨85⟩:** Meets the requirements
- **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products ⟨1⟩*.

## ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers and protect from light. Store at controlled room temperature.
- **USP Reference Standards ⟨11⟩**
  USP Folic 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 FOR INJECTION | Documentary Standards Support | SM32020 Small Molecules 3 |
| REFERENCE STANDARD SUPPORT | RS Technical Services<br>RSTECH@usp.org | SM32020 Small Molecules 3 |

**Chromatographic Database Information:** Chromatographic Database

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