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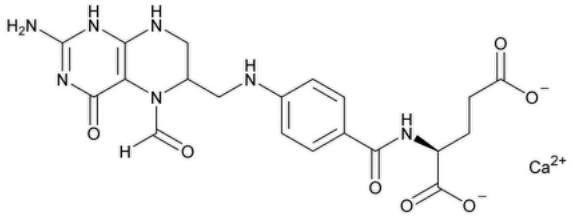
# Leucovorin Calcium



$C_{20}H_{21}CaN_7O_7$ 511.50

L-Glutamic acid, *N*-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt (1:1);

Calcium [4-({[(*RS*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl}amino)benzoyl]-L-glutamate CAS RN®: 1492-18-8; UNII: RPR1R4C0P4.

## DEFINITION

Leucovorin Calcium contains NLT 95.0% and NMT 105.0% of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$), calculated on the anhydrous basis.

## IDENTIFICATION

***Change to read:***

• **A.** ▲▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197**▲ (CN 1-May-2020) [Note—Methods described in ⟨197K⟩ or ⟨197A⟩ may be used.]▲ (USP 1-Aug-2019)

Do not dry specimens.

**Acceptance criteria:** Meets the requirements

***Add the following:***

▲• **B.** **Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Calcium*:** Meets the requirements▲ (USP 1-Aug-2019)

***Add the following:***

▲• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

## ASSAY

***Change to read:***

• **Procedure**

▲▲ (USP 1-Aug-2019) 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:** 1.0 M 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 a 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.

**System suitability stock solution:** 0.175 mg/mL of USP Folic Acid RS in *Diluent*

**Standard solution:** 0.175 mg/mL of USP Leucovorin Calcium RS in *Diluent*

**Sample solution:** 0.2 mg/mL of Leucovorin Calcium 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; ▲5-µm▲ (USP 1-Aug-2019) packing L1

**Flow rate:** ▲1.5 mL/min▲ (USP 1-Aug-2019)

**Injection volume:** 15 µL

▲**Run time:** 2.5 times the retention time of the leucovorin peak▲ (USP 1-Aug-2019)

**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 ▲▲ (USP 1-Aug-2019) and folic acid

**Relative standard deviation:** NMT 2.0% ▲for the leucovorin peak▲ (USP 1-Aug-2019)

**Analysis**

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

Calculate the percentage of leucovorin calcium ($C_{20}H_{21}CaN_7O_7$) in the portion of Leucovorin Calcium 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 Leucovorin Calcium RS in the *Standard solution* (mg/mL)

$C_U$ = concentration of Leucovorin Calcium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0% on the anhydrous basis

## IMPURITIES

***Add the following:***

▲• **ORGANIC IMPURITIES**

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

**Buffer:** Dissolve 2.6 mL of tetrabutylammonium hydroxide solution (40% in water) and 2.8 g of disodium hydrogen phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 7.8.

**Mobile phase:** Methanol and *Buffer* (150:850)

**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

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

**Sample solution:** 1.1 mg/mL of Leucovorin Calcium 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

**Run time:** 4 times the retention time of leucovorin

**System suitability**

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

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

$r_U$ = 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)

$C_U$ = concentration of Leucovorin Calcium in the *Sample solution* (mg/mL)

$F$ = 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 | 0.5 |
| 4-Aminobenzoylglutamic acid[b] | 0.59 | 1.0 | 1.5 |
| 10-Formyldihydrofolic acid[c] | 0.76 | 0.47 | 1.0 |
| 5,10-Diformyltetrahydrofolic acid[d] | 0.85 | 0.49 | 1.5 |
| Leucovorin | 1.0 | 1.0 | — |
| 7,8-Dihydrofolic acid[e] | 1.15 | 1.0 | 0.5 |
| 10-Formylfolic acid[f] | 2.11 | 0.60 | 0.5 |
| Folic acid | 2.50[g] | 1.0 | 1.5 |
| Individual, unspecified impurity | — | 1.0 | 0.3 |
| Total impurities[h] | — | — | 2.5▲ (USP 1-Aug-2019) |

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

[h] Includes all impurities in *Table 1* and 5-(γ-folinoyl)tetrahydrofolate from the test for *Limit of 5-(γ-Folinoyl)tetrahydrofolate* if the latter is possible from the manufacturing process.

***Add the following:***

▲• **Limit of 5-(γ-Folinoyl)tetrahydrofolate** (if present)

The chemical name of 5-(γ-folinoyl)tetrahydrofolate is {4-[({(S)-2-amino-5-[(S)-4-(4-{[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino}benzamido)-4-carboxybutanoyl]-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl}methyl)amino]benzoyl}-L-glutamic acid.

**Solution A:** Acetonitrile

**Solution B:** Methanol

**Solution C:** Dissolve 3.25 g of tetrabutylammonium hydroxide solution (40% in water) and 1.36 g of potassium dihydrogen phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 7.0.

**Mobile phase:** See *Table 2*.

**Table 2**

| Time (min) | Solution A (%) | Solution B (%) | Solution C (%) |
|---|---|---|---|
| 0 | 1.5 | 4.0 | 94.5 |
| 32 | 1.5 | 15.0 | 83.5 |
| 60 | 1.5 | 35.5 | 63.0 |
| 65 | 1.5 | 4.0 | 94.5 |
| 70 | 1.5 | 4.0 | 94.5 |

**Standard solution:** 1.0 mg/mL of USP Leucovorin Calcium RS in water

**Sample solution:** 1.0 mg/mL of Leucovorin Calcium in water

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 287 nm

**Column:** 2.1-mm × 10-cm; 3.5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 0.3 mL/min

**Injection volume:** 5 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for leucovorin and 5-(γ-folinoyl)tetrahydrofolate are 1.0 and about 1.7, respectively.]

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of 5-(γ-folinoyl)tetrahydrofolate in the portion of Leucovorin Calcium taken:

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

$r_U$ = peak response of 5-(γ-folinoyl)tetrahydrofolate from the *Sample solution*

$r_T$ = sum of all the peak responses from the *Sample solution*

**Acceptance criteria:** NMT 0.3%. It is included in the *Total impurities* in *Table 1* if it is possible from the manufacturing process.▲ (USP 1-Aug-2019)

## SPECIFIC TESTS

• **WATER DETERMINATION ⟨921⟩, *Method I*:** NMT 17.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

***Change to read:***

• **USP REFERENCE STANDARDS ⟨11⟩**

▲USP Folic Acid RS▲ (USP 1-Aug-2019)

USP Leucovorin Calcium RS

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

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

**Chromatographic Database Information:** Chromatographic Database

**Most Recently Appeared In:**
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