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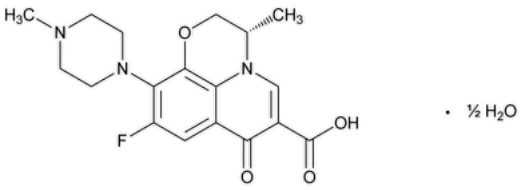
# Levofloxacin



• ½ $H_2O$

$C_{18}H_{20}FN_3O_4 \cdot ½H_2O$ 370.38

7*H*-Pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-hydrate (2:1), (*S*)-; (−)-(*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid, hemihydrate CAS RN®: 138199-71-0; UNII: 6GNT3Y5LMF.

Anhydrous CAS RN®: 100986-85-4.

## DEFINITION

Levofloxacin contains NLT 98.0% and NMT 102.0% of $C_{18}H_{20}FN_3O_4$, calculated on the anhydrous basis.

## IDENTIFICATION

• **A.** **Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**

• **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**

**Buffer:** 8.5 g/L of ammonium acetate, 1.25 g/L of cupric sulfate, pentahydrate, and 1.3 g/L of L-isoleucine in water

**Mobile phase:** Methanol and *Buffer* (3:7)

**Standard solution:** 1 mg/mL of USP Levofloxacin RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Levofloxacin in *Mobile phase*

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 360 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 45°

**Flow rate:** 0.8 mL/min

**Injection size:** 25 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** 0.5–1.5

**Relative standard deviation:** NMT 1.0%

**Analysis**

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

Calculate the percentage of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Levofloxacin taken:

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

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

$r_S$ = peak response of levofloxacin from the *Standard solution*

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

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

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

• **Residue on Ignition ⟨281⟩:** NMT 0.2%. Use a platinum crucible.

***Change to read:***

• **Organic Impurities, Procedure 1**

[Note—*Procedure 1* is recommended if levofloxacin *N*-oxide is a potential organic impurity. *Procedure 2* and *Procedure 3* are recommended if levofloxacin related compound B is a potential organic impurity.]

▲**Buffer,**▲ (ERR 1-Feb-2022) **Mobile phase, Sample solution,** and **Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 1 mg/mL of USP Levofloxacin RS in *Mobile phase*

**Sensitivity solution:** 0.3 µg/mL of USP Levofloxacin RS in *Mobile phase*

**System suitability**

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

**Suitability requirements**

**Relative standard deviation:** NMT 1.0%, *System suitability solution*

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

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each individual impurity in the portion of Levofloxacin taken:

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

$r_U$ = peak response of each impurity

$r_S$ = peak response of levofloxacin

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

**Acceptance criteria:** See *Table 1*.

**Table 1**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| *N*-Desmethyl levofloxacin[a] | 0.47 | 1.0 | 0.3 |
| Diamine derivative[b] | 0.52 | 0.9 | 0.3 |
| Levofloxacin *N*-oxide[c] | 0.63 | 1.1 | 0.3 |
| 9-Desfluoro levofloxacin[d] | 0.73 | 1.0 | 0.3 |
| Levofloxacin | 1.0 | — | — |
| D-Isomer[e] | 1.23 | 1.0 | 0.8 |
| Any unknown impurity | — | 1.0 | 0.1 |
| Total Impurities | — | — | 0.5* |

* Do not include the D-isomer in the calculation for total impurities.

[a] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

[b] (*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

[c] (*S*)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-10-yl)-1-methyl-piperazine-1-oxide.

[d] (*S*)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

[e] (*R*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

• **Organic Impurities, Procedure 2**

[Note—Solutions of levofloxacin are not stable in light; use amber bottles.]

**Buffer:** Dissolve 3.08 g/L of ammonium acetate and 8.43 g/L of sodium perchlorate monohydrate in water. Adjust with phosphoric acid to a pH of 2.2.

**Solution A:** Acetonitrile and *Buffer* (16:84)

**Solution B:** Acetonitrile, methanol, and *Buffer* (30:20:50)

**Solution C:** 0.4 mg/mL of USP Levofloxacin RS by dissolving in acetonitrile at about 8% of volume and diluting with water to volume

**Solution D:** 0.05 mg/mL of USP Levofloxacin Related Compound A RS in 0.2% ammonium hydroxide in methanol

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

**Table 2**

| Time (min) | Solution A (%) | Solution B (%) |
|---|---|---|
| 0 | 100 | 0 |
| 5 | 100 | 0 |
| 10 | 82 | 18 |
| 15 | 40 | 60 |
| 30 | 40 | 60 |
| 30.1 | 100 | 0 |
| 38 | 100 | 0 |

**System suitability solution:** 0.1 mg/mL of USP Levofloxacin RS and 5 µg/mL of USP Levofloxacin Related Compound A RS in water from *Solution C* and *Solution D*

**Levofloxacin stock solution:** 0.4 mg/mL of USP Levofloxacin RS. Dissolve USP Levofloxacin RS in acetonitrile at about 8% of final volume, sonicate, and dilute with water to volume.

**Levofloxacin standard solution:** 0.02 mg/mL of USP Levofloxacin RS in acetonitrile and water (1:10) from *Levofloxacin stock solution*

**Levofloxacin related compound B stock solution:** 0.2 mg/mL USP Levofloxacin Related Compound B RS in methanol. [NOTE—Sonicate if necessary.]

**Levofloxacin related compound B standard solution:** 0.04 mg/mL USP Levofloxacin Related Compound B RS in methanol from *Levofloxacin related compound B stock solution*

**Standard solution:** 0.4 µg/mL of levofloxacin and 0.8 µg/mL of levofloxacin related compound B in acetonitrile and water (1:10) from *Levofloxacin standard solution* and *Levofloxacin related compound B standard solution*

**Sample solution:** 0.4 mg/mL by dissolving the sample in acetonitrile at about 8% of final volume and diluting with water to volume. [NOTE—Sonicate if necessary.]

**Chromatographic system**

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

**Mode:** LC

**Detector:** 280 nm

**Column:** 4.0-mm × 15-cm; 3.0-µm packing L1

**Column temperature:** 38°

**Flow rate:** 1.0 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0% for levofloxacin

**Analysis**

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

Calculate the percentage of levofloxacin related compound B in the portion of Levofloxacin taken:

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

$r_U$ = peak response for levofloxacin related compound B from the *Sample solution*

$r_S$ = peak response for levofloxacin related compound B from the *Standard solution*

$C_S$ = concentration of USP Levofloxacin Related Compound B RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of other impurities in the portion of Levofloxacin taken:

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

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

$r_S$ = peak response of levofloxacin from the *Standard solution*

$C_S$ = concentration of USP Levofloxacin RS in the *standard solution* (mg/mL)

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

**Acceptance criteria:** See *Table 3*.

**Table 3**

| Name | Relative Retention Time | Acceptance Criteria, NMT (%) |
|---|---|---|
| Levofloxacin related compound A (*N*-Desmethyl levofloxacin)[a] | 0.9 | 0.20 |
| Levofloxacin | 1.0 | — |
| Levofloxacin related compound B[b] | 2.9 | 0.13 |
| Any other impurity | — | 0.10 |
| Total impurities | — | 0.50 |

[a] (*S*)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4-benzoxazine-6-carbocylic acid.

[b] (*S*)-9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4-benzoxazine-6-carbocylic acid.

- **Organic Impurities (Enantiomeric Purity), Procedure 3**

**Buffer:** 1.32 g/L of D-phenylalanine and 0.75 g/L of copper(II)sulfate pentahydrate in water

**Mobile phase:** Methanol and *Buffer* (15:85)

**System suitability solution:** 0.01 mg/mL of USP Ofloxacin RS and 0.01 mg/mL of USP Levofloxacin RS in water

**Sample solution:** 0.08 mg/mL in water

**Chromatographic system**

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

**Mode:** LC

**Detector:** 294 nm

**Column:** 4.6-mm × 15-cm; 3.5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 0.7 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *System suitability solution*

[Note—The relative retention times for D-ofloxacin and levofloxacin are 0.91 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between D-ofloxacin (D-isomer) and levofloxacin

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of D-ofloxacin in the portion of Levofloxacin taken:

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

$r_U$ = peak response for D-ofloxacin

$r_T$ = sum of responses of all peaks

**Acceptance criteria:** NMT 1.0%

**SPECIFIC TESTS**

• **Optical Rotation,** *Specific Rotation* ⟨781S⟩

**Solvent:** Methanol

**Sample solution:** 5 mg/mL in *Solvent*

**Acceptance criteria:** −92° to −106°, at 20°

• **Water Determination,** *Method Ia*⟨921⟩**:** 2.0%–3.0%

**ADDITIONAL REQUIREMENTS**

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at room temperature.

• **Labeling:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure the article complies.

• **USP Reference Standards** ⟨11⟩

USP Levofloxacin RS

USP Levofloxacin Related Compound A RS

(*S*)-9-Fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

$C_{17}H_{18}FN_3O_4$ 347.34

USP Levofloxacin Related Compound B RS

(*S*)-9,10-Difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid.

$C_{13}H_9F_2NO_4$ 281.21

USP Ofloxacin RS

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

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| LEVOFLOXACIN | Documentary Standards Support | SM12020 Small Molecules 1 |

**Chromatographic Database Information:** Chromatographic Database

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