

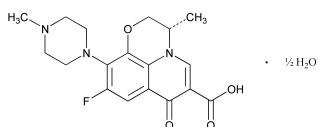
BRIEFING

Levofloxacin. This USP Pending Monograph was authorized as Version 1 on June 11, 2007. A proposed revision to include the chemical names for levofloxacin related compounds A and B in the test for *Related compounds* was posted on the USP website for public comment for more than 90 days. No comments were received. The MD-AA Expert Committee approved this revision to be incorporated into the Authorized USP Pending Monograph. The HPLC procedures used in the test for *Related compounds* and in the *Assay* are based on analysis performed with the YMC-OD-A brand of L1 column. The HPLC procedure used in the test for *Enantiomeric purity* is based on analysis performed with the Symmetry Shield RP18 brand of L1 column.

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Levofloxacin

v.2 Authorized February 1, 2009



$C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$ 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 [138199-71-0].
Anhydrous 361.37 [100986-85-41].

» Levofloxacin contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{20}FN_3O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, and store at room temperature.

USP Reference standards (11)—*USP Levofloxacin RS*. *USP Levofloxacin Related Compound A RS*. *USP Levofloxacin Related Compound B RS*. *USP Ofloxacin RS*.

Identification—

A: *Infrared Absorption* (197K).
B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Water, Method I (921): between 2.0% and 3.0%.

Residue on ignition (281): not more than 0.1%. [NOTE—Use a platinum crucible.]

Heavy metals, Method II (231): 0.001%.

Related compounds—[NOTE—Solutions of Levofloxacin are not stable in light; use amber bottles.]

Solution A, Solution B, Mobile phase, and System suitability preparation—Prepare as directed in the *Assay*.

Levofloxacin stock solution—Dissolve an accurately weighed quantity of about 10 mg of USP Levofloxacin RS in 2 mL of acetonitrile, sonicate, and quantitatively dilute with water to 25 mL. Transfer 5 mL of this solution to a 100-mL volumetric flask, dilute with a mixture of water and acetonitrile (10 : 1) to volume, and mix.

Levofloxacin related compound B stock solution—Dissolve an accurately weighed quantity of about 10 mg of USP Levofloxacin Related Compound B RS in methanol, sonicate, and quantitatively dilute with methanol to 50 mL. Transfer 2 mL of this solution to a 10-mL volumetric flask, dilute with methanol to volume, and mix.

Standard solution—Transfer 2 mL each of *Levofloxacin stock solution* and *Levofloxacin related compound B stock solution* into the same 100-mL volumetric flask, dilute with a mixture of water and acetonitrile (10 : 1) to volume, and mix.

Test solution—Transfer about 10 mg of Levofloxacin, accurately weighed, to a 25-mL volumetric flask, dissolve in 2 mL of acetonitrile, sonicate, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—Proceed as directed in the *Assay*, except to program the chromatograph as shown in *Table 1*.

Table 1

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–5	100	0	isocratic
5–10	100→82	0→18	linear gradient
10–15	82→40	18→60	linear gradient
15–30	40	60	isocratic
30–30.1	40→100	60→0	step gradient
30.1–38	100	0	re-equilibration

Procedure—Separately inject equal volumes (about 10 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of levofloxacin related compound B in the portion of Levofloxacin taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Levofloxacin Related Compound B RS in the *Standard solution*; C_U is the concentration, in mg per mL, of Levofloxacin in the *Test solution*; r_U is the peak response for levofloxacin related compound B obtained from the *Test solution*; and r_S is the peak response for levofloxacin related compound B obtained from the *Standard solution*. Calculate the percentage of other levofloxacin related compounds or impurities in the portion of Levofloxacin taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Levofloxacin RS in the *Standard solution*; C_U is the concentration, in mg per mL, of Levofloxacin in the *Test solution*; r_U is the peak response for levofloxacin related compounds obtained from the *Test solution*; and r_S is the peak response for levofloxacin obtained from the *Standard solution*. The limits of related compounds or impurities meet the requirements specified in *Table 2*.

Table 2

Related Compound/ Impurity	Relative Retention Time	Limit (%)
A ¹	0.9	0.20
B ²	2.9	0.13
Any other impurity	—	0.1
Total impurities	—	0.50

¹ (*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.

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

Enantiomeric purity—

Buffer solution—Dissolve 1.32 g of D-phenylalanine and 0.75 g of copper(II)sulfate pentahydrate in about 500 mL of water, dilute with water to 1000 mL, and mix.

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Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution* and methanol (85 : 15). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

System suitability solution—Dissolve accurately weighed quantities of USP Ofloxacin RS and USP Levofloxacin RS in and dilute with water to obtain a solution having a final concentration of about 0.01 mg of each per mL.

Test solution—Transfer about 20 mg of Levofloxacin, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 2 mL into a 10-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 294-nm detector and a 4.6-mm × 15-cm column that contains 3.5-μm packing L1. The flow rate is about 0.7 mL per minute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between D-ofloxacin and levofloxacin is not less than 2.0. [NOTE—The relative retention times are about 0.91 for D-ofloxacin and 1.0 for levofloxacin.]

Procedure—Inject about 10 μL of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for all the peaks. Calculate the percentage of D-ofloxacin in the portion of Levofloxacin taken by the formula:

$$100(r_i/r_s)$$

in which *r_i* is the response for each impurity; and *r_s* is the sum of the responses of all the peaks; not more than 1.0% of D-ofloxacin is found.

Assay—

Buffer solution—Dissolve 3.08 g of ammonium acetate and 8.43 g of sodium perchlorate monohydrate in about 500 mL of water, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 2.2, filter, and degas. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

Solution A—Prepare a mixture of *Buffer solution* and acetonitrile (84 : 16).

Solution B—Prepare a mixture of *Buffer solution*, acetonitrile, and methanol (50 : 30 : 20).

Solution C—Transfer about 10 mg of USP Levofloxacin RS to a 25-mL volumetric flask, dissolve in 2 mL of acetonitrile, dilute with water to volume, and mix.

Solution D—Transfer about 5.0 mg of USP Levofloxacin Related Compound A RS to a 100-mL volumetric flask, dissolve in and dilute with 0.2% ammonium hydroxide in methanol to volume, and mix.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system* (see *Table 3*).

Table 3

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–5	100	0	isocratic
5–10	100→82	0→18	linear gradient
10–15	82→40	18→60	linear gradient
15–15.1	40→100	60→0	step gradient
15.1–20	100	0	re-equilibration

System suitability preparation—Transfer 5 mL of *Solution C* and 2 mL of *Solution D* to a 20-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Levofloxacin RS in 2 mL of acetonitrile, sonicate, and dilute quantitatively, and stepwise if necessary, with a mixture of water and acetonitrile (10 : 1) to obtain a solution having a known concentration of about 0.02 mg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Levofloxacin in 2 mL of acetonitrile, sonicate, and dilute quantitatively, and stepwise if necessary, with a mixture of water and acetonitrile (10 : 1) to obtain a solution having a known concentration of about 0.02 mg per mL.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 15-cm column that contains 3.0-μm packing L1. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 38°. The chromatograph is programmed as shown in *Table 3*. Chromatograph the *System suitability preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between levofloxacin related compound A and levofloxacin is not less than 3.0; and the relative standard deviation for replicate injections for levofloxacin is not more than 2.0%. [NOTE—The relative retention times are about 0.9 for levofloxacin related compound A and 1.0 for levofloxacin.]

Procedure—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of C₁₈H₂₀FN₃O₄ in the portion of Levofloxacin taken by the formula:

$$100(C_s/C_u)(r_u/r_s)$$

in which *C_s* and *C_u* are the concentrations, in mg per mL, of levofloxacin in the *Standard preparation* and the *Assay preparation*, respectively; and *r_u* and *r_s* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.