

BRIEFING

Levofloxacin, page 347 of *PF* 32(2) [Mar.–Apr. 2006]. This monograph was published in *PF* for public review and comment before the establishment of the USP Pending Standards Web page. The MD-AA Expert Committee reviewed the comments submitted by the monograph sponsor and has approved the monograph as an Authorized USP Pending Standard. The following is a summary of the comments and the Expert Committee's decisions:

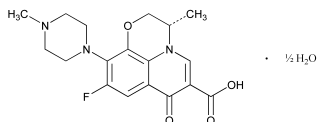
1. Comment Summary 1: Commenter suggested that the formula for the *Assay* calculation be reformatted to include concentrations because the *Standard preparation* and the *Assay preparation* indicate only the final concentration. Response: Comment incorporated.
2. Comment Summary 2: Commenter suggested the use of platinum crucibles in the *Residue on ignition* test because the fluorine in Levofloxacin will react with the porcelain of silica and may cause bias for quantitation of the inorganic residues. Response: Comment incorporated.
3. Comment Summary 3: Several comments with limited data were received suggesting that the specification/procedures for the *Assay* and the *Related compounds* test be changed to meet other manufacturer's product specification and criteria. Response: Because multiple products may have different specifications and impurity profile, the use of different criteria will be considered in the future with more supporting validation data either in a flexible monograph or an official *USP* monograph if the product has been fully approved by FDA.
4. Comment Summary 4: Commenter suggested using D-phenylalanine rather than D-phenylamine as the reagent in the preparation of the *Buffer solution* in the *Enantiomeric purity* test. Response: Comment incorporated.
5. Comment Summary 5: Commenter suggested that because the working medium in the Karl Fischer (KF) water determination test contains solvents (trichloromethane or chloroform), it should not be used due to potential solvent exposure and health risk. Response: The Expert Committee decided not to incorporate the comment because modern KF units are very well closed. Potential exposure to humans occurs during cleaning and refilling of the unit, and at those times the analyst needs to be wearing the proper personal protective equipment.

(MD-AA: B. Davani) RTS—43750

Add the following:

■ **Levofloxacin**

v.1 Authorized June 11, 2007



$C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$ 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)	<i>Solution A</i> (%)	<i>Solution B</i> (%)	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 µL) 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:

$$100(C_s/C_v)(r_v/r_s)$$

in which C_s is the concentration, in mg per mL, of USP Levofloxacin Related Compound B RS in the *Standard solution*; C_v is the concentration, in mg per mL, of Levofloxacin in the *Test solution*; r_v 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:

$$100(C_s/C_v)(r_v/r_s)$$

in which C_s is the concentration, in mg per mL, of USP Levofloxacin RS in the *Standard solution*; C_v is the concentration, in mg per mL, of Levofloxacin in the *Test solution*; r_v 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	0.9	0.20
B	2.9	0.13
Any other impurity	—	0.1
Total impurities	—	0.50

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.

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)	<i>Solution A</i> (%)	<i>Solution B</i> (%)	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 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. ■