Impurity Table 1 *(Continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
D-Isomer[e]	1.23	1.0	0.8
Any unknown impurity	—	1.0	0.1

[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.

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**⟩**:** 2.1%–2.7%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight and light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Levofloxacin RS

Add the following:

▲Levofloxacin Oral Solution

DEFINITION

Levofloxacin Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$).

IDENTIFICATION

- **A.** 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**
  [NOTE—Protect the solutions of levofloxacin from light.]
  Diluent: Acetonitrile and water (18:82)
  Mobile phase: *Diluent* that contains 1 mL of trifluoroacetic acid in each 1000 mL of solution
  Standard solution: 102.5 µg/mL of USP Levofloxacin RS in *Diluent*
  System suitability solution: 102.5 µg/mL each of USP Levofloxacin RS and USP Levofloxacin Related Compound A RS in *Diluent*
  Sample solution: 102.5 µg/mL of levofloxacin in *Diluent* based on the label claim. [NOTE—Mix the solution well after equilibrating the solution for 4 h at room temperature while protected from light.]
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 294 nm
  Column: 4.6-mm × 15-cm; 3.5-µm packing L11
  Column temperature: 30°
  Flow rate: 0.7 mL/min
  Run time: 2.5 times the retention time of the levofloxacin peak
  Injection size: 20 µL
  System suitability
  Samples: *Standard solution* and *System suitability solution*
  Suitability requirements
  Resolution: NLT 1.9 between levofloxacin related compound A and levofloxacin, *System suitability solution*
  Relative standard deviation: NMT 2.0%, *Standard solution*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$) in the portion of Oral Solution 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 Levofloxacin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

- **PROCEDURE**
  [NOTE—Protect the solutions of levofloxacin from light.]
  Diluent, Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of each individual impurity in the portion of Oral Solution taken:

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

r_U = peak response of each individual 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 = nominal concentration of levofloxacin in the *Sample solution* (mg/mL)
F = relative response factor for each impurity (See *Impurity Table 1*)

Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
9-Desfluoro levofloxacin[a]	0.64	1.0	—*
Diamine derivative[b]	0.75	1.0	—*
Levofloxacin related compound A[c]	0.91	0.81	0.5
Levofloxacin	1.0	—	—

[a] (*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.
[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*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carbocylic acid.
[d] (*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-methylpiperazine 1-oxide.
* Disregard this peak because this is a process impurity controlled for the drug substance.

Impurity Table 1 *(Continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levofloxacin *N*-oxide[d]	1.55	0.93	0.5
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

[a] (*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.
[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*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carbocylic acid.
[d] (*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-methylpiperazine 1-oxide.
***** Disregard this peak because this is a process impurity controlled for the drug substance.

SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/mL, and the total combined molds and yeast count does not exceed 10^1 cfu/mL. It also meets the requirement for absence of *Escherichia coli*.
- **DELIVERABLE VOLUME** ⟨**698**⟩**:** Meets the requirements
- **pH** ⟨**791**⟩**:** 5.0–6.0

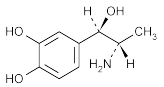
ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Store at controlled room temperature, and protect from light.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Levofloxacin RS
7H-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.
Anhydrous.
$C_{18}H_{20}FN_3O_4 \cdot {}^1/_2H_2O$ 370.38

USP Levofloxacin Related Compound A RS
(*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-carbocylic acid.
$C_{17}H_{18}FN_3O_4$ 347.34▲*USP35*

Levonordefrin



$C_9H_{13}NO_3$ 183.20
1,2-Benzenediol, 4-(2-amino-1-hydroxypropyl)-, [*R*-(*R**,*S**)]-.
(-)-α-(1-Aminoethyl)-3,4-dihydroxybenzyl alcohol [18829-78-2; 829-74-3].

» Levonordefrin, dried in vacuum at 60° for 15 hours, contains not less than 98.0 percent and not more than 102.0 percent of $C_9H_{13}NO_3$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Levonordefrin RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—
Solution: 25 µg per mL.
Medium: 0.1 N hydrochloric acid.

Specific rotation ⟨781S⟩: between −28° and −31°.

Test solution: 50 mg, previously dried, per mL, in 0.3 N hydrochloric acid.

Loss on drying ⟨731⟩**—**Dry it in vacuum at 60° for 15 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Chromatographic purity—

Standard solutions—Dissolve an accurately weighed quantity of USP Levonordefrin RS in a mixture of methanol and glacial acetic acid (96:4) to obtain a Standard stock solution having a known concentration of 5 mg per mL. Dilute this solution quantitatively with a mixture of methanol and glacial acetic acid (96:4) to obtain *Standard solutions*, designated below by letter, having the following compositions:

Standard solution	Dilution	Concentration (µg RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 10)	500	1.0
B	(1 in 20)	250	0.5
C	(1 in 50)	100	0.2
D	(1 in 100)	50	0.1

Test solution—Dissolve an accurately weighed quantity of Levonordefrin in a mixture of methanol and glacial acetic acid (96:4) to obtain a solution containing 50 mg per mL.

Procedure— Apply separately 5 µL of the *Test solution* and 5 µL of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of *n*-butyl alcohol, water, and glacial acetic acid (70:20:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wavelength UV light. Expose the plate to iodine vapors, and examine again. Compare the intensities, observed by both visualizations, of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions:* the sum of the intensities of secondary spots obtained from the *Test solution* corresponds to not more than 1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

Assay—Transfer about 350 mg of Levonordefrin, previously dried and accurately weighed, to a small flask, dissolve in 50 mL of glacial acetic acid, heating, if necessary, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 18.32 mg of $C_9H_{13}NO_3$.