Revision of the Monograph on

LEVOFLOXACIN

Draft proposal for *The International Pharmacopoeia*

(January 2019)

*DRAFT FOR COMMENTS*

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SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/17.717:

REVOLUTION OF THE MONOGRAPH ON LEVOFLOXACIN

DRAFT PROPOSAL FOR THE INTERNATIONAL PHARMACOPOEIA

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<td>Monograph drafted.</td>
<td>March 2017</td>
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<td>Discussion at the informal consultation on quality control laboratory tools and specifications for medicines.</td>
<td>2–4 May 2017</td>
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<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations.</td>
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<td>Laboratory investigations to verify the suitability of the methods and specifications.</td>
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<td>Draft revision sent out for public consultation.</td>
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<td>Further follow-up action as required.</td>
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[Note from the Secretariat. It is proposed to revise the monograph on Levofloxacin. The revision is based on an evaluation of information found in other pharmacopoeias, in the scientific literature and on laboratory investigations performed by a collaborating laboratory.]
Levofloxacin
(Levofloxacín)
Identity test

Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from levofloxacin RS or with the reference spectrum of levofloxacin hemihydrate.

B. Carry out the test as described under 1.14.1. Thin layer chromatography, using silica gel R5 as the coating substance and a mixture of 4 volumes of 1-butanol R, 4 volumes of methanol R and 2 volumes of ammonia (~100 g/L) TS as the mobile phase. Apply separately to the plate 5 μl of each of the following two solutions in a mixture of 1 volume of methanol R and 4 volumes of dichloromethane R. For solution (A), use a solution containing 5 mg of the test substance per mL. For solution (B), use a solution containing 5 mg of levofloxacin RS per mL. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (366 nm).

The principal spot obtained with solution (A) corresponds in position, appearance, and intensity with the spot due to levofloxacin in the chromatogram obtained with solution (B).

C. Dissolve 25 mg of the test substance in about 20 ml of hydrochloric acid (~4 g/l) TS and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml using water R. The absorption spectrum (1.6) of the resulting solution, when observed between 210 and 350 nm, exhibits two maxima at about 227 nm and at about 294 nm.

D. Carry out test D.1 or D.2.

D.1 Determine the specific optical rotation (1.4) using a solution containing 5.0 mg of the test substance per mL methanol R and calculate with reference to the anhydrous substance; \([\alpha]^D_{20} = -92\) to \(-106\).

D.2 Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Related substances”. The area of
any peak corresponding to impurity A is not greater than ten times the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (1.0 %).

**Heavy metals.** Use 2.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 μg/g.

**Sulfated ash (2.3).** Not more than 1.0 mg/g.

**Water.** Determine as described under 2.8 Determination of water by Karl Fischer Method, Method A. Use 0.500 g of the test substance. The water content is not less than 20 mg/g and not more than 30 mg/g.

**Related substances.** Prepare fresh solutions protected from light and perform the test without delay.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm), packed with end-capped and base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 μm).¹

Prepare the following buffer solution. Dissolve 1.25 g of copper (II) sulfate pentahydrate R, 1.3 g of isoleucine R and 8.5 g ammonium acetate R in water R and dilute to 100 mL with the same solvent.

As the mobile phase, use a mixture of methanol R and the buffer solution (30:70 v/v). Operate with a flow rate of 0.8 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 360 nm. Maintain the column at a temperature of 45 °C.

Prepare the following solutions in mobile phase. For solution (1), dissolve 50.0 mg of the test substance in 50.0 mL. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 1.0 mL of this solution to 10.0 mL. For solution (3), use a solution containing 1.0 mg of levofloxacin for system suitability RS (containing levofloxacin and the impurities A, B and G) per mL.

¹ Inertsil ODS-2 or ODS-3 columns were found suitable.
Inject alternately 25 µL of solution (1), (2) and (3). Record the chromatogram for three times the retention time of levofloxacin.

Use the chromatogram obtained with solution (3) and the chromatogram supplied with levofloxacin for system suitability RS to identify the peaks due to the impurities A, B and G. The impurities are eluted, if present, at the following relative retention with reference to levofloxacin (retention time about 20 minutes); impurity B about 0.50; impurity G about 0.56; impurity A about 1.22.

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity B and the peak due to impurity G is at least 1.5.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.3, is not greater than three times the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (0.3 %);
- the area of any peak corresponding to impurity G, when multiplied by a correction factor of 1.2, is not greater than three times the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (0.3 %);
- the area of any peak corresponding to impurity A is not greater than ten times the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (1.0 %);
- the area of any other impurity peak is not greater than the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (0.10 %);
- the sum of the corrected areas of any peak corresponding to impurity B or G and the areas of all other impurity peaks, other than any peak due to impurity A, is not greater than five times the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (0.5 %). Disregard any peak with an area less than 0.5 times the area of the peak due to levofloxacin in the chromatogram in the chromatogram obtained with solution (2) (0.05%).

**Impurity F.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Related substances” with the following modifications.
As the mobile phase, use a mixture of methanol R and the buffer solution (50:50 v/v). As a detector, use an ultraviolet spectrophotometer set at a wavelength of 320 nm.

Prepare the following solutions in mobile phase. For solution (1), dissolve 50.0 mg of the test substance in 50.0 mL. For solution (2), dissolve 5.0 mg of levofloxacin impurity F RS and dilute to 100.0 mL. For solution (3), dilute 4.0 mL of solution (2) to 100.0 mL. For solution (4), dilute 4.0 mL of solution (2) to 10.0 mL. Dilute 1.0 ml of this solution to 10.0 mL with solution (1).

Inject alternately 25 µL of solution (1), (3) and (4). Record the chromatogram for three times the retention time of levofloxacin.

Use the chromatogram obtained with solution (3) to identify the peaks due to impurity F. Impurity F, if present, is eluted at the relative retention of 1.8 with reference to levofloxacin (retention time: about 6 minutes).

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution between the peaks due to impurity F and the peak due to levofloxacin is at least 5.

Measure the areas of the peaks corresponding to impurity F obtained in the chromatograms of solution (1) and (3) and calculate the percentage content of impurity F. The concentration of impurity F is not more than 0.2%.

**Assay.** Prepare fresh solutions protected from light and perform the test without delay.

Dissolve about 0.300 g, accurately weighed, in 100 ml of glacial acetic acid R and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6. *Non-aqueous titrations*, Method A determining the end point potentiometrically. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.14 mg of \( \text{C}_{18}\text{H}_{20}\text{FN}_{3}\text{O}_{4} \).

**Impurities**
A. (3R)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1, 4-benzoaxazine-6-carboxylic acid (dextrofl Roxacin, synthesis-related impurity),

B. (3S)-9-fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoaxazine-6-carboxylic acid (N-desmethyl levofloxacin),

C. 4-[(3S)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoazin-10-yl]-1-methylpiperazine 1-oxide (levofloxacin N-oxide; degradation product),

D. (3S)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoaxazine-6-carboxylic acid (9-desfluoro levofloxacin, synthesis related impurity),

E. (3S)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoaxin-7-one (decarboxy levofloxacin, synthesis related impurity),
F. (3S)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoazine-6-carboxylic acid,

G. (3S)-9-fluoro-3-methyl-10-[(2-(methylamino)ethyl)amino]-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (diamine derivative)

H. ethyl (3R)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylate

I. (3S)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (levofloxacin 9-piperazino isomer, synthesis related impurity)
New reference substances to be established

Levofloxacin RS
ICRS to be established.

Levofloxacin impurity F RS
ICRS to be established.

Levofloxacin for system suitability RS (containing levofloxacin and the impurities A, B and G)
It is intended to refer to the corresponding reference substance to be established for the European Pharmacopoeia.

New reagent to be added

Ammonia (~10 g/L) TS
Ammonia (~100 g/L) TS, diluted to contain about 10 g of NH₃ per litre (approximately 1% (w/v)).

Copper (II) sulfate pentahydrate R
CuSO₄·5H₂O, [7758-99-8]; blue, crystalline powder or transparent, blue crystals, content: 99.0% to 101.0%.

L-Isoleucine R
(2S,3S)-2-Amino-3-methylpentanoic acid, C₆H₁₃NO₂, content: 98.5% to 101.0% (dried substance).