

LEVOFLOXACIN
DRAFT PROPOSAL FOR
THE INTERNATIONAL PHARMACOPOEIA
(SEPTEMBER 2010)

REVISED DRAFT FOR COMMENT

This document was provided by a quality control expert and was discussed at the recent WHO consultation on specifications for medicines and quality control laboratory issues. Previous comments received have been incorporated into this revised draft. Should you have any comments, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mails: kopps@who.int with a copy to Ms C. Mendy mendyc@who.int by 3 November 2010.

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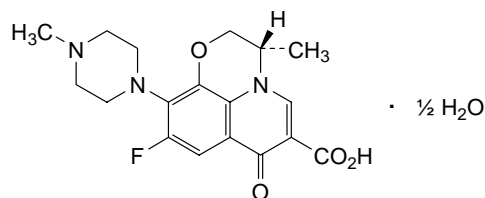
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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/10.360
International Pharmacopoeia monograph on Levofloxacin

	Date
Preparation of first draft by laboratory	April-May 2010
Discussion at consultation on specifications for medicines and quality control laboratory issues	10-12 May 2010
Draft monograph mailed out for comments	July 2010
Collation of comments	August 2010
Comments discussed during video-/teleconference on specifications for medicines	25 August 2010
Revised draft mailed out for comments	October 2010
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations	18-22 October 2010
Further action as necessary	...

Draft proposal for *The International Pharmacopoeia*
(September 2010)

LEVOFLOXACIN



C₁₈H₂₀FN₃O₄ · ½ H₂O

Relative Molecular Mass. 370.4

Chemical name. (*S*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid hemihydrate; CAS Reg. No. 138199-71-0.

Description. Yellowish white to bright yellow, crystals or crystalline powder.

Solubility. Slightly soluble in water, soluble in glacial acetic acid, slightly soluble or soluble in dichloromethane, slightly soluble in methanol.

Category. Antibacterial.

Storage. Levofloxacin should be kept in a tightly closed container, protected from light.

Requirements

Definition. Levofloxacin contains not less than 99.0% and not more than 101.0% of levofloxacin (C₁₈H₂₀FN₃O₄) calculated with reference to the anhydrous substance.

Manufacture. The production method is validated to ensure that the substance is the *S*-enantiomer.

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.

- B. Carry out the test as described under 1.14.1. Thin layer chromatography, using silica gel R6 as the coating substance and a mixture of 10 volumes of dichloromethane R, 5 volumes of methanol R and 1 volume of ammonia solution 1% 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 5 mg of Levofloxacin per ml. For solution (B) use 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 (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

- C. Transfer 25 mg of Levofloxacin to a 50-ml volumetric flask. Add about 20 ml of hydrochloric acid (~4 g/l) TS, sonicate for about 5 minutes, allow to cool to room temperature and make up to the volume using the same solvent. Filter a portion of this solution through a 0.45-µm filter, discarding the first few ml of the filtrate. 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 294 nm and at about 327 nm. The specific absorbance ($A_{1\text{cm}}^{1\%}$) at 294 nm is between 876 and 948.
- D. Determine the specific optical rotation (1.4) using a 30 mg/ml solution dissolved in a mixture of 10 volumes of methanol R and 40 volumes of dichloromethane R and calculate with reference to the anhydrous substance; $[\alpha]^{20^\circ\text{C}} = -12^\circ$ to -11° .

[Note from Secretariat: suitability of carrying out the Optical rotation test in methanol under investigation and corresponding limits to be confirmed.]

Heavy metals

[Note from Secretariat: suitable test for heavy metals under investigation.]

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 1.0 g of the test substance. The water content is not less than 21 mg/g and not more than 27 mg/g.

Impurity A

Carry out the test as described under 1.14.1. Thin layer chromatography, using silica gel R6 as the coating substance and a mixture of 10 volumes of glacial acetic acid R, 10 volumes of water R and 20 volumes of ethyl acetate R. Apply separately to the plate 10 µl of each of the two following solutions in the dissolution solvent prepared by mixing 10 volumes of methanol R and 40 volumes of dichloromethane R. For solution (A) use 50 mg of Levofloxacin per ml. For solution (B) use 0.1 mg of levofloxacin impurity A RS per ml. After removing the plate from the chromatographic chamber, allow to dry in air. Examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A corresponding impurity A is not more intense than the principal spot obtained with solution B.

Other related substances

Prepare fresh solutions, protected from light and perform the tests without delay.

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm), packed with particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm)¹.

Maintain the column temperature at 45°C.

Prepare the mobile phase as follows: dissolve 4.0 g of ammonium acetate R and 7.0 g of sodium perchlorate R in water R and dilute to 1300 ml; adjust to pH 2.2 with phosphoric acid R and add 240 ml of acetonitrile R.

Prepare the following solutions in the dissolution solvent prepared in mixing 10 volumes of acetonitrile R and 60 volumes of water R.

For solution (1) dissolve 10 mg of Levofloxacin in the dissolution solvent and dilute to 50.0 ml with the same solvent. For solution (2) dilute 1.0 ml of solution (1) to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with the same solvent. For solution (3) dissolve 10 mg of levofloxacin impurity E RS in the dissolution solvent and dilute to 100.0 ml with the same solvent. Mix 10 ml with 5 ml of solution (1) and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with the same solvent.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 294 nm.

Inject 20 µl of solution (3). The test is not valid unless the resolution factor between the peaks due to impurity E and Levofloxacin is at least 2.

Inject separately 20 µl each of solutions (1), (2) and of the dissolution solvent in the chromatographic system.

In the chromatogram obtained with solution (1), the following impurity peaks, if present, are eluted at the following relative retention with reference to Levofloxacin (retention time about 17 minutes): impurity B about 0.36; impurity C about 0.57; impurity D about 0.75; impurity E about 0.91; impurity F about 1.50.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 2.6, is not greater than the area of the principal peak obtained with solution (2) (0.2%);
- the area of any peak corresponding to impurity D, when multiplied by a correction

¹ Symmetry 150 x 4.6 mm (5 µm) is suitable.

- factor of 4.2, is not greater than the area of the principal peak obtained with solution (2) (0.2%);
- the area of any peak corresponding to impurity C, E or F is not greater than the area of the principal peak obtained with solution (2) (0.2%);
 - the area of any other impurity peak is not greater than 0.5 times the area of the principal peak obtained with solution (2) (0.1%);
 - the sum of the areas (corrected, where necessary) of all the peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak obtained with solution (2) (0.05%).

[*Note from Secretariat: following information to be confirmed:*

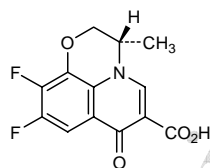
- *correction factors for impurities B and D,*
- *limit for individual unspecified impurities,*
- *limit for total of impurities]*

Assay

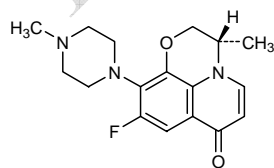
Dissolve about 0.300 g, accurately weighed, in 100 ml of glacial acetic acid and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6. Non aqueous titrations, Method A determining the end point potentiometrically. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.14 mg of $C_{18}H_{20}FN_3O_4$.

Impurities

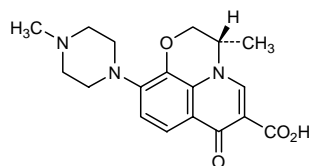
The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information:



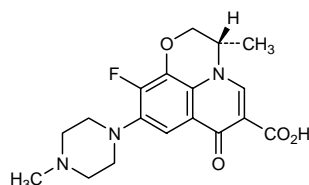
A. (3*S*)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



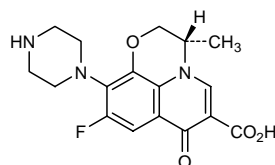
B. (3*S*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazin-7-one,



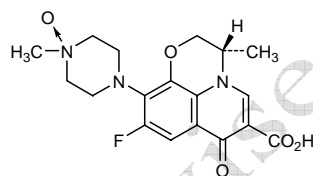
C. (3*S*)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



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



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



F. 4-[(3*S*)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazin-10-yl]-1-methylpiperazine 1-oxide.

New reagent to be added to Ph.Int

Hydrochloric acid (~4 g/l) TS.

Dilute 10 ml of hydrochloric acid (~420 g/l) TS with sufficient water to produce 1000 ml (approximately 0.1 mol/l).
