

LEVOFLOXACIN TABLETS
DRAFT PROPOSAL FOR
THE INTERNATIONAL PHARMA COPOEIA
(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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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/10.362
International Pharmacopoeia monograph on Levofloxacin tablets

	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
Revised draft discussed during a 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 TABLETS

Category. Antibacterial.

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

Labelling. The designation of the container of Levofloxacin tablets should state that the active ingredient is the hemihydrate form and the quantity should be indicated in terms of the equivalent amount of Levofloxacin.

Additional information. Strengths in the current WHO Model list of essential medicines: 200 mg, 400 mg. Strengths in the current WHO Model list of essential medicines for children: 200 mg, 400 mg.

Requirements

Comply with the monograph for "Tablets".

Definition. Levofloxacin tablets contain Levofloxacin. They contain not less than 90.0% and not more than 110.0% of the amount of Levofloxacin ($C_{18}H_{20}FN_3O_4$) stated on the label.

Identity test

- Either test A alone or any two of tests B, C and D may be applied
- A. To a quantity of the powdered tablets containing 100 mg of Levofloxacin, add 10 ml of acetonitrile R, shake, filter and evaporate to dryness. 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 μ l of each of the two following solutions in a mixture of 1 volume of methanol R and 4 volumes of dichloromethane R. For solution (A) shake a quantity of the powdered tablets containing 25 mg of Levofloxacin with 5 ml, filter and use the clear filtrate. 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. See the test described under Assay method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).
- D. The absorption spectrum of the final solution prepared for Assay method B, when observed between 210 and 350 nm, exhibits two maxima at about 294 nm and at about 327 nm.

[Note from Secretariat: a specific optical rotation test to differentiate levofloxacin from ofloxacin is under investigation, with the possibility to include such test under a Manufacture section.]

Dissolution test

Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 ml of hydrochloric acid (~ 4 g/l) TS, and rotating the paddle at 100 revolutions per minute. At 30 minutes withdraw a sample of about 5 ml of the medium through an in-line filter. Measure the absorbance (1.6) of a 1-cm layer of the filtered sample at the maximum at about 294 nm. At the same time, measure the absorbance at the maximum at about 294 nm of a suitable solution of levofloxacin RS in hydrochloric acid (~ 4 g/l) TS using hydrochloric acid (~ 4 g/l) TS as a blank.

For each of the six tablets, calculate the total amount of Levofloxacin ($C_{18}H_{20}FN_3O_4$), in the medium. The amount in solution for each tablet is not less than 75% of the amount declared on the label. If the amount obtained for one of the six tablets is less than 75%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 70% and the amount obtained for no tablet is less than 55%.

[Note from Secretariat: dissolution conditions and limits to be confirmed]

Related substances

Prepare fresh solutions and perform the tests without delay. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay, method A.

For solution (1) transfer a quantity of the powdered tablets containing about 10 mg of Levofloxacin into about 20 ml of the dissolution solvent, sonicate for 5 minutes, allow to cool to room temperature and dilute to 50.0 ml with the same solvent. Filter a portion of this solution through a 0.45- μ m filter, discarding the first few ml of the filtrate. 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.

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 greater than 2.

Inject separately 20 µl each of solutions (1) and (2) and of the dissolution solvent in the chromatographic system. Examine the dissolution solvent chromatogram for any extraneous peaks and disregard the corresponding peaks observed in the chromatogram obtained with solution (1).

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 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: as for the API monograph, following information to be confirmed:

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

Assay

- Either method A or method B may be applied.
- A. Prepare fresh solutions 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 by mixing 10 volumes of acetonitrile R and 60 volumes of water R.

For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 20 mg of Levofloxacin, accurately weighed, into about 20 ml of the dissolution solvent, sonicate for 5 minutes, allow to cool to room temperature and dilute to 50.0 ml with the same solvent. Filter a portion of this solution through a 0.45-µm filter, discarding the first few ml of the filtrate. Dilute 5 ml of this solution to 25 ml with the dissolution solvent.

For solution (2) dissolve 2.0 mg of ofloxacin RS in the dissolution solvent and dilute to 25.0 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) and (2) and of the dissolution solvent in the chromatographic system..

In the chromatogram obtained with solution (1), the following peaks 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.

Measure the areas of the peak responses in the chromatograms obtained with solutions (1) and (2). Calculate the content of Levofloxacin (C₁₈H₂₀FN₃O₄).

- B. Weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 25 mg of Levofloxacin, accurately weighed, to a 50-ml volumetric flask. Add about 20 ml of hydrochloric acid (~4 g/l) TS, sonicate for about 5 minutes, allow to

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

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. Measure the absorbance (1.6) of a 1-cm layer of this solution at the maximum at about 294 nm. Calculate the content of Levofloxacin in the tablets using an absorptivity value of 91.2 ($A_{1\%}^{1\text{cm}} = 912$).

Impurities. The impurities limited by the requirements of this monograph include impurities B to F listed in the monograph for Levofloxacin.

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).

Revised draft for comment