Consultation documents

The International Pharmacopoeia

REVISION OF THE MONOGRAPH ON ETHINYLESTRADIOL (ETHINYLESTRADIOLUM)

Draft proposal for inclusion in The International Pharmacopoeia (December 2018)

DRAFT FOR COMMENTS

Please send any comments you may have on the attached text to Dr Herbert Schmidt, Technical Officer, Medicines Quality Assurance, Technologies Standards and Norms (schmidt@who.int), with a copy to Ms Sinead Jones (jonessi@who.int) by 28 February 2019.

Medicines Quality Assurance working documents will only be sent out electronically and will also be placed on the Medicines website for comment under “Current projects”. If you have not already received our draft working documents, please send your email address to jonessi@who.int and we will add your name to our electronic mailing list.

Note from the Secretariat. It is proposed to revise the monograph on Ethinylestradiol as follows:

- Replace the existing TLC method to test for related substances with an HPLC method.
- Add an alternative assay method.
- Add an alternative identity test C by HPLC and revise the identity test B by TLC.
- Add a transparency list to the monograph.

The proposed changes are based on information found in the European Pharmacopoeia and in Kommentar zum Europäischen Arzneibuch, Gesamtwerk mit 53. Aktualisierungslieferung 2016, Wissenschaftliche Verlagsgesellschaft Stuttgart.

Changes from the current monograph are indicated in the text by insert or delete.
Draft proposal for inclusion in The International Pharmacopoeia

REVISION OF THE MONOGRAPH ON
ETHINYLESTRADIOL
(ETHINYLESTRADIOLUM)

Ethinylestradiol (Ethinylestradiolum)

Molecular formula. C$_{20}$H$_{24}$O$_2$

Relative molecular mass. 296.4

Graphic formula.

![Chemical structure of Ethinylestradiol]

Chemical name. 19-Nor-17$\alpha$-pregna-1,3,5(10)-trien-20-yn-3,17-diol; 17-ethynylestra-1,3,5,(10)-triene-3,17$\beta$-diol; CAS Reg. No. 57-63-6.

Description. A white to slightly yellowish white, crystalline powder; odourless.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS; soluble in acetone R, and dioxan R and dilute alkaline solutions.

Category. Estrogen.

Storage. Ethinylestradiol should be kept in a well-closed container, protected from light.

Additional information. Ethinylestradiol may exhibit polymorphism, may exist in 2-polymorphic forms one of which melts at about 183°C, the other, metastable, at about 143°C.

Requirements

Definition. Ethinylestradiol contains not less than 97.5-97.0% and not more than 102.0% of C$_{20}$H$_{24}$O$_2$, calculated with reference to the dried substance.
Identity tests

- Either test A or tests B and C 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 ethinylestradiol RS or with the reference spectrum of ethinylestradiol.

If the spectrum thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and ethinylestradiol RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from ethinylestradiol RS. If the spectrum obtained from the solid state of the test substance is not concordant with the spectrum obtained from the reference substance, compare the spectra of solutions in chloroform R containing 30 mg/mL, using a path length of 0.2 mm.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R1 as the coating substance and a mixture of 10 volume of dehydrated ethanol R and 90 volumes of toluene R as the mobile phase. Apply separately to the plate 5 μL of each of two solutions in a mixture of 10 volumes of methanol R and 90 volumes of dichloromethane R containing (A) 1.0 mg of the test substance per mL, and (B) 1.0 mg of ethinylestradiol RS per mL. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to air dry until the solvents have evaporated, heat at 110 °C for 10 minutes, spray the hot plate with sulfuric acid/ethanol (20%) TS and heat again at 110 °C for 10 minutes. Allow to cool and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution (A) corresponds in position, appearance, and intensity with that obtained with solution (B). Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 1 volume of propylene glycol R and 9 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use toluene R as the mobile phase. Apply separately to the plate 2 μL of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 1.0 mg of the test substance per mL, and (B) 1.0 mg of ethinylestradiol RS per mL. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120°C for 15 minutes, spray with 4-toluenesulfonic acid/ethanol TS, and then heat at 120°C for 5–10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.
C. Carry out the test as described under 1.14.4 *High-performance liquid chromatography* using the conditions and solutions given under “Assay”, Method A. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to ethinylestradiol in the chromatogram obtained with solution (5).

**Specific optical rotation.** Use a 4.0 mg/mL solution in pyridine R and calculate with reference to the dried substance; \([\alpha]_{D}^{20^\circ} = -27.0^\circ \text{ to } -30.0^\circ.\]

**Loss on drying.** Dry to constant weight at 105°C; it loses not more than 10 mg/g.

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

Use the following conditions for gradient elution:

**mobile phase A:** 30 volumes of acetonitrile for chromatography R and 70 volumes of water R;

**mobile phase B:** 25 volumes of water R and 75 volumes of acetonitrile for chromatography R.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–35</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>35–65</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>65–66</td>
<td>0 to 100</td>
<td>100 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>66–75</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>
Prepare the following solutions using a mixture of 40 volumes of water R and 60 volumes of acetonitrile R as diluent. For solution (1), dissolve 50.0 mg of the test substance in 30 mL of acetonitrile and dilute to 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), dissolve 2 mg of estrone R (impurity C) in 10.0 mL. Dilute 1.0 mL of this solution to 100.0 mL. For solution (4), dissolve the content of a vial of ethinylestradiol for system suitability RS (containing ethinylestradiol and the impurities B, F, H, I and K) in 1.0 mL of solution (3).

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 220 nm. Maintain the column temperature at 30 °C. Inject alternatively 30 µL each of solution (1), (2) and (4) and record the chromatograms.

Use the chromatogram obtained with solution (4) and the chromatogram supplied with ethinylestradiol for system suitability RS to identify the peaks due to the impurities B, C, F, H, I and K. The impurities, if present, are eluted at the following relative retention with reference to ethinylestradiol (retention time about 35 min): impurity F about 0.2; impurity H about 0.5; impurity I about 0.8; impurity B about 0.88; impurity C about 0.92; impurity K about 1.3.

The test is not valid unless in the chromatogram obtained with solution (4) the resolution between the peaks due to impurity I and B is at least 1.2.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 0.7, is not greater than five times the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.5 %);
- the area of any peak corresponding to impurity I, when multiplied by a correction factor of 0.4, is not greater than twice the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurity H or K is not greater than twice the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.2 %);
- the area of any peak corresponding to impurity C or F is not greater than 1.5 times the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.15 %);
- the area of any other impurity peak is not greater than the area of the peak due to ethinylestradiol in the chromatogram obtained with solution (2) (0.10 %);
• the sum of the corrected areas of any peak corresponding to impurity B and I
and the areas of all other impurity peaks is not greater than eight times the
area of the peak due to ethinylestradiol in the chromatogram obtained with
solution (2) (0.8 %). Disregard any peak with an area less than 0.5 times the
area of the peak due to ethinylestradiol in the chromatogram obtained with
solution (2) (0.05 %).

Estrone. Carry out the test as described under 1.14.1 Thin-layer chromatography, using
silica gel R1 as the coating substance and a mixture of 92 volumes of dichloroethane R, 8
volumes of methanol R, and 0.5 volumes of water as the mobile phase. Apply separately to
the plate 5 μl of each of 2 freshly prepared solutions in a mixture of 9 volumes of chloroform
R and 1 volume of methanol R containing (A) 20 mg of the test substance per mL, and (B).
0.20 mg of estrone RS per mL. After removing the plate from the chromatographic
chamber, allow it to dry in air until the odour of the solvent is no longer detectable; then
heat at 110°C for 10 minutes. Spray the hot plate with sulfuric acid/ethanol TS, heat again
at 110°C for 10 minutes, and examine the chromatogram in ultraviolet light (365 nm). The
spot obtained with solution B is more intense than any spot, corresponding in position and
appearance, obtained with solution A.

Assay

• Either method A or method B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid
chromatography, using the conditions given below under “Related substances”.

Use solution (1) as described under “Related substances”. Prepare the following
additional solution: for solution (5), dissolve 50.0 mg of ethinylestradiol RS in 30 mL
of acetonitrile R and dilute to 50.0 mL with water R.

Inject alternately 50 μL each of solution (1) and (5) and record the chromatograms.

Measure the areas of the peaks corresponding to ethinylestradiol obtained in the
chromatograms of solutions (1) and (5) and calculate the percentage content of
ethinylestradiol (C_{20}H_{24}O_{2}) using the declared content of C_{20}H_{24}O_{2} in ethinylestradiol
RS.

B. Dissolve 50.0 mg of the test substance in sufficient dehydrated ethanol R and dilute
to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 50.0 mL with
the same solvent.

Measure the absorbance of a 1 cm layer of the diluted solution at the maximum at
about 281 nm. Calculate the percentage content of ethinylestradiol (C_{20}H_{24}O_{2}) using
the absorptivity value of 7.1 (A_{1%}^{\text{cm}} = 71).
Dissolve about 0.05 g, accurately weighed, in sufficient dehydrated ethanol R to produce 100 mL, and dilute 10.0 mL of this solution to 50.0 mL with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 281 nm. Calculate the amount of C_{20}H_{24}O_{2} in the substance being tested by comparison with ethinylestradiol RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.72 ± 0.04.

Impurities

A. **19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol (17β-ethinylestradiol)**

B. **19-nor-17α-pregna-1,3,5(10),9(11)-tetaen-20-yne-3,17-diol (degradation product)**
C. 3-hydroxyestra-1,3,5(10)-trien-17-one (estrone) (synthesis related impurity, degradation product)

D. estra-1,3,5(10)-triene-3,17β-diol (estradiol) (degradation product)

E. 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,6α,17-triol (6α-hydroxy-ethinylestradiol) (degradation product)
F. 19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,6β,17-triol (6β-hydroxy-ethinylestradiol) (degradation product)

G. 3,17-dihydroxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-6-one (6-oxo-ethinylestradiol) (degradation product)

H. 3,17-dihydroxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-16-one (16-oxo-ethinylestradiol)
I. 19-nor-17α-pregna-1,3,5(10),6-tetraen-20-yn-3,17-diol

J. 1-methyl-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,17-diol (1-methyl-ethinylestradiol)

K. 4-methyl-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,17-diol (4-methyl-ethinylestradiol)
L.  
\textit{estra-1,3,5(10)-triene-3,17\alpha\text{-}diol (17\alpha\text{-}estradiol)}

M.  
\textit{2-methyl-19-nor-17\alpha\text{-}pregna-1,3,5(10)-trien-20-yne-3,17-diol (2-methyl-ethinylestradiol)}
Consultation documents

The International Pharmacopoeia

POLYMORPHISM

Draft chapter for The International Pharmacopoeia
(December 2018)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft to Dr Herbert Schmidt, Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland; email: schmidth@who.int, with a copy to Ms Sinéad Jones (email: jonessi@who.int) by 28 February 2019.

In order to speed up the process for receiving draft monographs and for sending comments, please send us your email address and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

Note from the Secretariat. It is proposed to publish the following chapter on Polymorphism in the Supplementary Information section under “Notes for guidance”.

The text was revised based on the comments received during the last public consultation in June – July 2018
POLYMORPHISM

1. INTRODUCTION AND TERMINOLOGY

The aim of this chapter is to provide a brief overview of:

- the terminology associated with crystal polymorphism;
- some analytical techniques commonly used to characterise polymorphs;
- the relevance of polymorphism for active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs); and
- the control strategies for polymorphism employed by The International Pharmacopoeia.

APIs and excipients, in the solid phase, can be classified as either crystalline or non-crystalline solids. A crystalline structure implies that the structural units (i.e. the unit cells) are repeated in a long range order (i.e. three dimensional crystal lattice). The arrangement of atoms and/or molecules in an amorphous solid is non-ordered (i.e. does not have a long range order), or random system, analogous to the liquid state, and does not possess a distinguishable crystal lattice. Amorphous solids are classified as non-crystalline solids.

Variation in the crystallization conditions (temperature, pressure, solvent composition, concentration, rate of crystallization, seeding of the crystallization medium, presence and concentration of impurities, etc.) may cause the formation of different crystalline forms.

When a chemical element (e.g. sulfur) exists in different crystalline forms, it is referred to as allotropy, not polymorphism (1). When a chemical compound with a given chemical structure crystallizes in more than one crystalline lattice with different unit cells, these crystalline phases are called polymorphs and the phenomena is referred to as polymorphism. The difference in internal crystal structure could be attributed to differences in molecule packing arrangements and/or different molecular conformations. Polymorphic substances, having identical chemical composition, will on dissolution exhibit the same chemical behaviour in solution.

Crystals of a given chemical compound with the same internal structure may exhibit different external shapes or crystal habits. In addition, variations in crystal habit may indicate the presence of polymorphism but is not necessarily indicative of polymorphic forms (12).

Solvates are crystal forms containing stoichiometric or non-stoichiometric quantities of a solvent. When the solvent incorporated into the crystal structure of the compound is water, the molecular adduct formed is referred to as a hydrate. Hydrates can be classified as three categories based on different structural aspects: Class I represents hydrates where the water molecules exist at isolated sites; Class II hydrates are generally referred to as channel hydrates; and Class III hydrates are generally referred to as ion-coordinated site hydrates. In such systems, water molecules form ion-water bonds that are usually much stronger than hydrogen bonds (13). Solvation and hydration products are also sometimes referred to as pseudopolymorphs (2, 3, 4). However, the term “pseudopolymorphism” is ambiguous because of its use in different circumstances. It is therefore preferable to use only the terms “solvates” and “hydrates”. 
Occasionally, a compound of a given hydration/solvation composition may crystallize into more than one crystalline form; an example of such a compound is nitrofurantoin (5). Nitrofurantoin can be crystallized as two monohydrate forms (Forms I and II) and two anhydrous forms (designated polymorphs α and β) (5).

Crystal forms are said to be isostructural (also referred to as isomorphous) when they have the same overall crystal packing. Solvates, which have the same overall crystal packing, but differ only in the solvents included in their crystal structures, are termed isostructural or isomorphous solvates, e.g. hydrate and isopropanolate of hexakis(2,3,6-tri-O-acetyl)-α-cyclodextrin (6).

The term desolvated solvate (or desolvated hydrates), also referred to as isomorphous desolvates, has been used to describe a solid form obtained by removing solvent from the solvate crystal structure (or water from a hydrate) without significantly changing the crystal structure (4), as in the desolvated monohydrate of terazosin HCl (7).

Amorphous forms of APIs and excipients are of substantial interest because they are usually more soluble (also having a faster kinetic solubility) than their crystalline counterparts but are thermodynamically less stable. Solid-state properties of amorphous forms of the same chemical compound (i.e. thermal behaviour, solubility profile, density, etc.) may differ.

Co-crystals are crystalline materials composed of two or more different molecules, typically an API and co-crystal formers (“coformers”) within the same crystal lattice that are associated by nonionic and noncovalent bonds. An example of a co-crystal is the succinic acid co-crystal of fluoxetine HCl (8). Co-crystals are thus more similar to solvates, in that both contain more than one component in the lattice. However, for co-crystals the coformer is non-volatile (i.e. exists as solid material at ambient conditions) (3).

Pharmaceutical co-crystals have gained considerable attention as alternative forms in an attempt to enhance the bioavailability, stability and processability of the API in the manufacturing process. Another advantage of co-crystals is that they generate a diverse array of solid state forms for APIs that lack ionisable functional groups, which is a prerequisite for salt formation (3). Guidance and reflection papers on the use and classification of pharmaceutical co-crystals have been published (3, 9).

2. CHARACTERIZATION AND THERMODYNAMIC STABILITY OF SOLID FORMS

Crystalline and amorphous forms are characterized based on their physicochemical properties. Table 1 lists some examples of the properties that may differ among different forms (9).
### Table 1. Examples of physicochemical properties that may differ among different forms

1. **Packing properties**
   a. Molar volume and density
   b. Refractive index
   c. Conductivity (electrical and thermal)
   d. Hygroscopicity

2. **Thermodynamic properties**
   a. Melting and sublimation temperatures
   b. Internal energy (i.e. structural energy)
   c. Enthalpy (i.e. heat content)
   d. Heat capacity
   e. Entropy
   f. Free energy and chemical potential
   g. Thermodynamic activity
   h. Vapour pressure
   i. Solubility

3. **Spectroscopic properties**
   a. Electronic state transitions
   b. Vibrational state transitions
   c. Nuclear spin state transitions

4. **Kinetic properties**
   a. Dissolution rate
   b. Rates of solid state reactions
   c. Stability
   d. Solid state

5. **Surface properties**
   a. Surface-free energy
   b. Interfacial tensions
   c. Habit (i.e. shape)

6. **Mechanical properties**
   a. Hardness
   b. Tensile strength
   c. Compactibility
   d. Flow
Table 2 summarizes some of the most commonly used techniques to study and/or classify different amorphous or crystalline forms. These techniques are often complementary and it is indispensable to use several of them. Demonstration of a non-equivalent structure by single crystal X-ray diffraction is currently regarded as the definitive evidence of polymorphism. X-ray powder diffraction and/or solid state NMR can also be used, as bulk techniques, to provide unequivocal proof of polymorphism (10).

Any technique(s) chosen to confirm the identity of the specific form(s) must be proven to be suitably specific for the identification of the desired form(s). Care must be taken in choosing the appropriate sample preparation technique, as heat generation, mechanical stress or exposure to elevated pressure and other environmental conditions (humidity) may trigger conversion between different forms.

Table 2. Examples of some techniques that may be used to study and/or classify different crystalline forms

| 1.  | X-ray powder diffraction* & Single crystal X-ray diffraction |
| 2.  | Microcalorimetry |
| 3.  | Thermal analysis (1.2.1 Melting point,* differential scanning calorimetry, thermogravimetry, thermomicroscopy) |
| 4.  | Moisture sorption analysis |
| 5.  | Polarized optical microscopy and electronic microscopy with diffraction capability (ex. Transmission Electron Microscopy) |
| 6.  | Solid-state nuclear magnetic resonance; |
| 7.  | Solubility studies |
| 8.  | Spectrophotometry in the infrared region (1.7)* and Raman spectrophotometry |
| 9.  | Intrinsic dissolution rate |
| 10. | Density measurement |

* Methods currently employed by The International Pharmacopoeia

Using suitable analytical techniques, the thermodynamic stability of the forms should be investigated. The form with the lowest free energy is the most thermodynamically stable at a given temperature and pressure. All other forms of the given system are in a metastable state. At standard temperature and pressure, a metastable form may remain unchanged or may change to a thermodynamically more stable form. In general, the more stable the form the less soluble it is. Conversion to a thermodynamically more stable form, may cause changes in some of the physicochemical properties (see Table 1) of the compound that may result in changes to other critical properties such as bioavailability, manufacturability (also referred to as processability), etc.

If there are several crystalline forms one form is thermodynamically more stable at a given temperature and pressure. A given crystalline form may constitute a phase that can reach equilibrium with other solid phases and with the liquid and gas phases.
If each crystalline form is stable within a given temperature range the change from one form to another is reversible and is said to be enantiotropic. The change from one phase to another is a univariate equilibrium so that at a given pressure this state is characterized by a transition temperature. However, if only one of the forms is stable over the entire temperature range, the change is irreversible or monotropic (11).

3. RELEVANCE OF POLYMORPHISM FOR APIs AND FPPs

Polymorphism (and hydrate formation) of APIs and excipients are of interest as they may affect bioavailability, toxicity and processability. Also, the thermodynamic stability of the form included in the FPP is considered important as environmental conditions may compromise the stability thereof. For formulations where the API is dissolved, attention has to be paid to supersaturation with regards to different forms. A formulation might not be supersaturated regarding a metastable polymorph but supersaturated with regards to the thermodynamically stable polymorph. Control of the form by the manufacturer may be required during the processing of APIs and excipients and during the manufacturing of a dosage form to ensure the correct physicochemical characteristics thereof. The control of a specific form is especially critical in the areas where the bioavailability, stability or processability are directly impacted (4).

The form of a readily soluble API that is incorporated into a solution, for example, an injection, an oral solution or eye drops, is normally non-critical (exceptions to this statement might be if the concentration of the solution is such that it is close to the limit of solubility of one of the possible polymorphs – as mentioned above - or solvate formation is observed with one of the excipients). Similarly, if an API is processed during the manufacturing process to obtain an amorphous form (e.g. hot melt extrusion, spray-dried dispersion, etc.), the original form is considered non-critical, as long as the processability is not influenced.

The form may be critical when the material is included in a solid dosage form or as a suspension in a liquid dosage form. In such cases, the characteristics of the different polymorphs may affect the bioavailability or dissolution of the material. The polymorphic form of a biopharmaceutics classification system (BCS) class I or III API in a solid oral dosage form is normally non-critical in terms of dissolution rate or bioavailability as by definition it would be readily soluble, but confirmation thereof by the manufacturer, is recommended. The ICH Harmonised Tripartite Guideline on Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances Q6A, provides guidance on when and how polymorphic forms should be controlled and monitored (4).

The inclusion of potentially harmful solvents in the crystal lattice, which may render APIs or excipients to be toxic or harmful to patients (i.e. solvates), should also be suitably regulated and monitored by the manufacturer.
4. POLYMORPHISM IN THE INTERNATIONAL PHARMACOPOEIA

Where a monograph indicates that a compound shows polymorphism this may be true crystal polymorphism, occurrence of solvates/hydrates or occurrence of the amorphous form.

The International Pharmacopoeia controls the polymorphic or crystalline forms (hereafter referred to as form) of a limited number of substances by restricting it to either:

- a single form, for example, carbamazepine API (Anhydrous Form III), mebendazole API (Form C); or
- by limiting the presence of unwanted forms, for example, chloramphenicol palmitate API (should contain at least 90% of polymorph B).

The control of forms specified in The International Pharmacopoeia may be achieved by:

- permitting no deviation from the infrared absorption spectrum of the reference substance prescribed (or reference spectrum supplied) – when the infrared absorption spectrum has been proven to be specific to the preferred form and able to distinguish the undesired form(s), for example, indomethacin API;
- restricting the melting point range, when the melting properties of the forms are clearly distinguishable, for example, phenobarbital API;
- recommending the use of any other suitable methods such as X-ray powder diffractometry, for example, carbamazepine tablets; and
- limiting the incorporated solvent (in the case of solvates/hydrates) with a specific limit test, for example, nevirapine hemihydrate API.

The specific control to be used will be indicated in the applicable monograph.

When the infrared identification test is able to detect differences in forms for a specific compound (i.e. polymorphism may be present for this compound), but the control of a specific form is not required by the monograph, the user may be instructed to:

- recrystallize both the test substance and the specified reference substance, in the event where the infrared spectra are found to be not concordant, for example, fluconazole API; and/or
- dry the API and/or specified reference substance to ensure that both forms are in the anhydrous or dehydrated state, for example, nevirapine hemihydrate API.

Whenever the choice of a specific form is critical with regard to bioavailability and/or stability, the method of the manufacturer of the product must be validated to consistently yield the desired polymorph in the final product at release and over its shelf life. The monograph will include a statement under the heading “Manufacturing” to draw attention to the control of a specified form during manufacturing where control is known to be critical, for example, carbamazepine oral suspension.
It is the intention of *The International Pharmacopoeia* to extend the inclusion of explicit statements in monographs, where appropriate, as information on the occurrence of polymorphism becomes available. The Secretariat thus cordially invites the users of *The International Pharmacopoeia* and manufacturers to share any relevant information that could be included in the monographs.

**References**


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Consultation documents

The International Pharmacopoeia

REVISION OF THE MONOGRAPH ON LEVOFLOXACIN

Draft proposal for The International Pharmacopoeia

(January 2019)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft to Dr Herbert Schmidt (schmidth@who.int), Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland, by 15 March 2019.

In order to speed up the process for receiving draft monographs and for sending comments, please send your email address to jonesi@who.int and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

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.
Levofloxacina

(Levofloxicinaum)

Molecular formula.  C_{18}H_{20}FN_{3}O_{4},\frac{1}{2}H_{2}O

Relative molecular mass.  370.4

Graphic formula.

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

Description.  Light yellowish-white or slightly yellow powder.

Solubility.  Sparingly soluble in water R, freely soluble in acetic acid (~300 g/L) TS, sparingly soluble in methanol R, and slightly soluble in dehydrated ethanol R.

Category.  Antibacterial, antituberculosis.

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

Requirements

Definition.  Levofloxacain contains not less than 98.0% and not more than 101.0% of levofloxacain (C_{18}H_{20}FN_{3}O_{4}) calculated with reference to the anhydrous substance.

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 levofloxacain RS or with the reference spectrum of levofloxacain 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; $\left[\alpha\right]_{D}^{20} = -92$ 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).\(^1\)

\(^1\) Inertsil ODS-2 or ODS-3 columns were found suitable.
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 1000 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.

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 C₁₈H₂₀FN₃O₄.
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-benzoxazine-6-carboxylic acid (dextrofloxacin, 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-benzoxazine-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-benzoxazin-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-benzoxazine-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-benzoxazin-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-benzoazaine-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).

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Consultation documents

The International Pharmacopoeia

REVISION OF THE MONOGRAPH ON LEVOFLOXACIN TABLETS

Draft proposal for The International Pharmacopoeia (January 2019)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft to Dr Herbert Schmidt (schmidth@who.int), Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland, by 31 March 2019.

In order to speed up the process for receiving draft monographs and for sending comments, please send your email address to jonesi@who.int and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

Note from the Secretariat. It is proposed to revise the monograph on Levofloxacin tablets.

The revision is based on and evaluation of information found in other pharmacopoeias, the scientific literature and on laboratory investigations performed by a collaborating laboratory.
Levofloxacin tablets
(Levofloxacini compressi)

**Category.** Antibacterial, antituberculosis.

**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 Levofloxacin (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 (EML): 250 mg, 500 and 750 mg. Strengths in the current WHO EML for children: 250 mg and 500 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 (C₁₈H₂₀FN₃O₄) stated on the label.

**Identity test**

Either test A or tests B and C may be applied.

A. To a quantity of the powdered tablets, nominally equivalent to 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 hemihydrate.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and levofloxacin RS in a small amount of acetonitrile R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from levofloxacin RS.
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 10 volumes of dichloromethane R, 5 volumes of methanol R and 1 volume of ammonia (~10 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), shake a quantity of the powdered tablets, nominally equivalent to 25 mg of levofloxacin, with 5 mL, filter and use the clear filtrate. 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 in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to levofloxacin in the chromatogram obtained with solution (B).

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to levofloxacin in the chromatogram obtained with solution (2).

Dissolution. 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 (~3.65 g/L) TS and rotating the basket at 100 revolutions per minute. At 30 minutes, withdraw a sample of about 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature and use is as solution (1). Measure the absorbance (1.6) of a 1 cm layer of the filtered sample at about 293 nm. At the same time, measure the absorbance 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 tablets, calculate the total amount of levofloxacin (C₁₈H₂₀FN₃O₄), in the medium. Each mg of levofloxacin hemihydrate (C₁₈H₂₀FN₃O₄,½ H₂O) is equivalent to 0.976 mg of levofloxacin (C₁₈H₂₀FN₃O₄).

Evaluate the results as described under 5.5 Dissolution test for solid oral dosage forms, Acceptance criteria. The amount of levofloxacin in solution for each tablet is not less than 80% (Q) of the amount declared on the label.

[Note from the Secretariat. It is intended to determine the absorptivity value of levofloxacin hemihydrate during the establishment of levofloxacin RS. The value will then be included in the test description.]
**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 the conditions given under “Assay”.

Prepare the following solutions in mobile phase. For solution (1), transfer a quantity of the powdered tablets, nominally equivalent to 250.0 mg of levofloxacin, into a 250 mL volumetric flask, add about 180 ml, sonicate for 5 minutes, dilute to volume, mix and filter. 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), dissolve 5.0 mg of levofloxacin for system suitability RS (containing levofloxacin and the impurities A, B and G) and dilute to 5.0 mL.

Inject 25 μL of solution (1), (2) and (3). Record the chromatogram for about three times the retention time of levofloxacin.

Use the chromatogram supplied with levofloxacin for system suitability RS and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B and G. The impurities, if present, are eluted at the following relative retentions with reference to levofloxacin (retention time about 20 minutes): impurity E about 0.38, impurity B about 0.50, impurity G about 0.56, impurity C about 0.63, impurity A about 1.22.

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks due to impurity B and 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 seven times the area of the peak due to levofloxacin in the chromatogram obtained solution with (2) (0.7%);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 1.47, is not greater than seven times the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (0.7%);
- the area of any peak corresponding to impurity E, when multiplied by a correction factor of 1.67, 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 1.20, 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 other impurity peak is not greater than twice the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (0.2%);

• the sum of the corrected areas of any peak corresponding to impurity B, C, E and G and the areas of all other impurity peaks, other than any peak due 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%). Disregard any peak with an area less the area of the peak due to levofloxacin in the chromatogram obtained with solution (2) (0.1%).

### Assay

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).\(^2\)

Prepare the following buffer solution. Dissolve 1.25 g of copper sulfate pentahydrate R, 1.3 g of isoleucine R and 8.5 g of ammonium acetate R in water R and dilute to 1000 mL with the same solvent.

As the mobile phase, use a mixture of methanol R and 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 temperature at 45 °C.

Weigh and powder 20 tablets. For solution (1), transfer a quantity of the powdered tablets, nominally equivalent to 50.0 mg of levofloxacin, into a 250 mL volumetric flask, add about 180 mL of the mobile phase, shake for 30 minutes, dilute to volume, mix and filter. For solution (2), dissolve 20.0 mg of levofloxacin RS in mobile phase and dilute to 100.0 mL using the same solvent. Inject alternately 10 μL each of solution (1) and (2) and record the chromatograms for about two times the retention time of levofloxacin.

Measure the areas of the peaks corresponding to levofloxacin obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of levofloxacin (C\(_{18}\)H\(_{20}\)FN\(_3\)O\(_4\)) in the tablets, using the declared content of C\(_{18}\)H\(_{20}\)FN\(_3\)O\(_4\) in levofloxacin RS.

### Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph for Levofloxacin.

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\(^2\) Inertsil ODS-2 or ODS-3 columns were found suitable.