Consultation Documents

The International Pharmacopoeia

Dissolution testing of tablets and capsules

Draft revision for inclusion in the supplementary information section of The International Pharmacopoeia (September 2013). Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidth@who.int. All working documents are posted for comment at http://www.who.int/medicines.

Introduction
The revision of chapter 5.5 Dissolution test for solid, oral dosage forms, as published in The International Pharmacopoeia*, was based on the internationally-harmonized dissolution test developed by the Pharmacopoeial Discussion Group (PDG) which comprises representatives from the European Pharmacopoeia, the Japanese Pharmacopoeia and the United States Pharmacopeia. The revised general method presents the Paddle and Basket methods for dissolution testing. Two other general methods contained in the PDG text, namely the Reciprocating-cylinder method and the Flow-through cell have not, so far, been adopted for The International Pharmacopoeia.

*Note from the Secretariat: It is intended to publish revised chapter 5.5 Dissolution test for solid, oral dosage forms, adopted in October 2012 at the 47th WHO Expert Committee on Specifications for Pharmaceutical Preparations, together with this text in the next supplement or edition of The International Pharmacopoeia.

It is not the intention of The International Pharmacopoeia to apply retrospectively the test conditions and acceptance criteria of the revised dissolution test or to change specifications for existing products. Table 1 lists monographs with dissolution tests, which were developed applying previous versions of chapter 5.5 and which are thus not subject to the internationally-harmonized provision. In the elaboration of new monographs and revision of individual monographs in The International Pharmacopoeia the principles of the revised test, e.g. to base acceptance criteria on “Q” values (dissolution limits), will be applied.

Table 1. Monographs on solid, oral dosage forms with dissolution test conditions and specifications elaborated before chapter 5.5 Dissolution test for solid, oral dosage forms, were revised to encompass the internationally-harmonized procedure.

- Amodiaquine tablets
- Artemether capsules
- Artemether tablets
Artemimol tablets
Artesunate tablets
Carbamazepine tablets
Chloroquine phosphate tablets
Chloroquine sulfate tablets
Doxycycline capsules
Doxycycline tablets
Efavirenz, emtricitabine and tenofovir tablets
Emtricitabine and tenofovir tablets
Emtricitabine capsules
Erythromycin ethylsuccinate tablets
Erythromycin stearate tablets
Ethambutol hydrochloride tablets
Griseofulvin tablets
Ibuprofen tablets
Indinavir capsules
Indometacin tablets
Isoniazid tablets
Isoniazid and ethambutol hydrochloride tablets
Levonorgestrel and ethinylestradiol tablets
Lopinavir and ritonavir tablets
Metronidazole tablets
Phenoxyacetylpenicillin potassium tablets
Phenytoin sodium tablets
Pyrazinamide tablets
Quinine bisulfate tablets
Quinine sulfate tablets
Rifampicin capsules
Rifampicin tablets
Ritonavir tablets
Saqquinavir tablets
Sulfadoxine and pyrimethamine tablets
Sulfamethoxazole and trimethoprim tablets
Tenofovir tablets

**Objective of dissolution testing**
While the ultimate objective of dissolution testing is to ensure adequate and reproducible bioavailability, the objective of the dissolution tests prescribed in the individual monographs of The International Pharmacopoeia is to obtain information about the drug-release characteristics of a particular formulation or batch of a product under standardized test conditions. Compliance with the test provides an assurance that most of the active ingredient will be dissolved in an aqueous medium within a reasonable amount of time when the preparation is subject to a mild agitation. Compliance with the dissolution test does not by itself guarantee bioavailability.

Standardized conditions and limits are considered appropriate for a pharmacopoeial test that is intended to apply to a monograph covering multisource products.

**Policy of The International Pharmacopoeia**
Monographs on tablet and capsule preparations listed in Table 1 include a dissolution test, either with or without further information on the test conditions. As a test method
spectrophotometry is typically employed. In case a dissolution test is prescribed an additional disintegration test is not required.

In the elaboration of new tablet and capsule monographs and revision of existing monographs decisions on dissolution and disintegration testing will be taken in agreement with the guidance given by the International Conference on Harmonisation (ICH) on the application of dissolution testing to medicinal products (see http://www.ich.org). The monograph will contain a dissolution test and/or a disintegration test. For rapidly dissolving (dissolution > 80% in 15 minutes at pH 1.2, 4.0 and 6.8) dosage forms containing active ingredients which are highly soluble throughout the physiological range (dose: solubility volume < 250 ml from pH 1.2 to 6.8), disintegration is substituted for dissolution. Disintegration is most appropriate when a relationship to dissolution has been established or when disintegration is shown to be more discriminating than dissolution.

When the disintegration test can be substituted for the dissolution test monographs on dosage forms will specify a choice between A (dissolution test) and B (disintegration test). If the disintegration requirement is not met dissolution testing has to be performed.

**Test conditions**

Following a decision made at the 45th Expert Committee meeting a standardized dissolution test is applied to conventional-release tablet and capsule formulations containing highly soluble active ingredients (Class I and III of the Biopharmaceutics Classification System (BCS)). The following conditions for a single-time test using the Paddle method are preferred:

- dissolution medium: dissolution buffer pH 6.8;
- volume of medium: 500 ml;
- rotation speed: 75 rpm;
- sampling time: 30 min.

When test conditions are not specified in the individual monograph it is recommended to apply similar test conditions. If the Basket method is used a rotation speed of 100 rpm is recommended.

For conventional-release tablet and capsule formulations containing poorly water-soluble active ingredients (Class II and IV of the BCS). (Classification of active ingredients included in the WHO Model List of Essential Medicines is provided by WHO in *Technical Reprt Series*, No. 937, Annex 7 (2006).) decisions on the appropriate test conditions are taken on a case-to-case basis. A single-point dissolution test is normally applied. Because of the low aqueous solubility dissolution medium of volume 900 ml and addition of a surfactant may be needed. The concentration of active ingredient at 100% dissolution should not exceed approximately 35% saturation.

For delayed-release dosage forms two-stage testing according to the procedure in 5.5 Dissolution test for solid, oral dosage forms is applied. It is important to consider the population of individuals who will be taking the dosage form when designing the test, e.g. administration of the dosage form to achlorhydric patients may require testing for resistance of the product against gastric juice at elevated pH, for example, pH 3.5.
For sustained-release dosage forms the appropriate test conditions and sampling procedures are specified in the monograph. Three time-points are applied.

**Acceptance criteria**

The revised dissolution test contains acceptance criteria for conventional-release, delayed-release and sustained-release dosage forms. The acceptance criteria are identical to those stated in the internationally harmonized dissolution test. The harmonized dissolution limits (Q-values) are applied in new and revised monographs (i.e., monographs on solid, oral dosage forms containing a dissolution test, but not listed in Table 1).

The three-level acceptance criteria, i.e. S1, S2 and S3 for conventional-release dosage forms, are not applied in monographs listed in Table 1; acceptance criteria for a two-stage test (6+6 dosage units) are specified in some monographs. For dosage forms for which the monograph require compliance with 5.5 Dissolution test for solid, oral dosage forms, but without specification of test conditions, it is recommended to apply a test using Q = 75% and the three-level acceptance criteria.

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**General monograph on parenteral preparations. Test for bacterial endotoxins (3.4)**

Draft for inclusion in the revised General Monograph on Parenteral Preparations in The International Pharmacopoeia (September 2013).

Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidt@who.int. All working documents are posted for comment at http://www.who.int/medicines.

During the forty-seventh meeting of the Expert Committee on Specifications for Pharmaceutical Preparations in October 2012 a revision of the general monograph on parenteral preparations was adopted.

One of the major changes to the monograph on parenteral preparations was the required compliance of all parenteral preparations with the test for bacterial endotoxins (or, where justified, pyrogens). As a consequence individual monographs on injectable dosage forms in The International Pharmacopoeia (Ph.Int.) were investigated with a view to add a limit for bacterial endotoxins to each monograph that currently does not include such a requirement.

The limits proposed are calculated using the following approaches (for details see Table 2).

- For the monograph on Ergometrine hydrogen maleate injection, the limit given in The International Pharmacopoeia for the respective pharmaceutical substance (for parenteral use) was applied to the strength of the respective dosage form listed in the WHO Model List of Essential Medicines (EML).

- For the monographs on Ephedrine sulfate injection, Magnesium sulfate injection, Oxytocin injection, Prednisolone sodium phosphate injection and Zidovudine intra-
venous infusion, the limits given in the respective monographs of the United States Pharmacopeia (USP) were taken and applied to the strength of the respective dosage forms listed in the EML.

- For the monographs on Artemether injection, Artemotil injection, Melarsoprol injection, Pentamidine isetionate powder for injections and Quinine dihydrochloride injection the endotoxin limits were calculated using recommendations given in Chapter 3.4 Test for bacterial endotoxins:

```markdown
<table>
<thead>
<tr>
<th>Ph.Int. monographs on parenteral preparations currently lacking limits for the bacterial endotoxins test</th>
<th>Proposed limits for the bacterial endotoxins test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Artemether injection</td>
<td>not more than 1.56 IU of endotoxin per mg Artemether</td>
</tr>
<tr>
<td>2  Artemotil injection</td>
<td>not more than 1.04 IU of endotoxin per mg Artemotil</td>
</tr>
<tr>
<td>3  Ephedrine sulfate injection</td>
<td>not more than 85 IU of endotoxin per ml</td>
</tr>
<tr>
<td>4  Ergometrine hydrogen maleate injection</td>
<td>not more than 140 IU of endotoxin per ml</td>
</tr>
<tr>
<td>5  Melarsoprol injection</td>
<td>not more than 50 IU of endotoxin per ml</td>
</tr>
<tr>
<td>6  Magnesium sulfate injection</td>
<td>not more than 45 IU of endotoxin per ml</td>
</tr>
<tr>
<td>7  Oxytocin injection</td>
<td>not more than 357 IU of endotoxin per ml</td>
</tr>
<tr>
<td>8  Pentamidine isetionate powder for injection*</td>
<td>not more than 1.25 IU of endotoxin per mg pentamidine isetionate</td>
</tr>
<tr>
<td>9  Prednisolone sodium phosphate for injection**</td>
<td>not more than 5.0 IU of endotoxin per mg prednisolone sodium phosphate</td>
</tr>
<tr>
<td>10 Quinine dihydrochloride injection</td>
<td>not more than 300 IU of endotoxin per ml</td>
</tr>
<tr>
<td>11 Zidovudine intravenous infusion</td>
<td>not more than 10 IU of endotoxin per ml</td>
</tr>
</tbody>
</table>

* The title of the monograph should also be changed to Pentamidine isetionate for injection.  
** The title of the monograph should also be changed to Prednisolone sodium phosphate for injection.
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the endotoxin limit for parenteral preparations, defined on the basis of dose, is equal to:

\[ \text{endotoxin limit} = \frac{K}{M} \]

K = threshold pyrogenic dose of endotoxin per kilogram of body mass (i.e. 5.0 IU per kg for any route of administration other than intrathecal)

M = maximum recommended bolus dose of product per kilogram of body mass. (When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period.)

In case the EML recommends different strengths of the same medicine the endotoxin limit is specified per mg active ingredient (see Artemether injection and Artemotil injection); in case only one strength is listed the limit is defined per ml (injection solution) (see Melarsoprol injection and Quinine dihydrochloride injections). For powders for injections the endotoxin limit is given per mg of active ingredient.

Additional changes
In the new general monograph on Parenteral preparations the statement is made that:

“For powders and concentrates for injections and intravenous infusions, the amount of the preparation to be tested and the nature and volume of the liquid in which it is to be dissolved, suspended or diluted is specified in the individual monograph.”

It is proposed to delete this sentence since the preparation of the sample solution is sufficiently described in Chapter 3.4. The harmonized text requires that the samples should be dissolved or diluted in aqueous solutions so that the final solutions do not exceed the maximum valid dilution (MVD).

In the monograph on Metronidazole injection the following provision for the test for bacterial endotoxins is made:

“Carry out the test as described under 3.4 Test for bacterial endotoxins. Dilute the injection, if necessary, with water LAL to give a solution containing 5 mg per ml (solution A). Solution A contains not more than 3.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.”

This sentence should be changed to read:

“Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 3.5 IU of endotoxin per ml.” Same rationale applies as given above.

For endotoxin limits already specified in The International Pharmacopoeia two phrases are used:

“… not more that x IU of endotoxin per mg/ml …”
“… not more that x IU of endotoxin RS per mg/ml …”
### Table 2. Data used in the calculation of bacterial endotoxin limits

<table>
<thead>
<tr>
<th>Drug</th>
<th>USP monograph</th>
<th>Endotoxin limits in respective USP monographs</th>
<th>Info on strength of dosage form (EML or PH.INT)</th>
<th>Info on dosage and route of application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemether injection</td>
<td>No USP monograph</td>
<td>17th EML: 80 mg/ml in 1-ml ampoule (oily injection); Ph.Int.: 80 mg/ml in 1-ml ampoule (oily injection), other available strengths: 40 mg/ml (paediatric formulation), 60 mg/ml, 100 mg/ml (adult formulation).</td>
<td>Intramuscular injection For adults, artesunate 2.4 mg/kg BW IV or IM given on admission (time = 0), then at 12 h and 24 h, then once a day is the recommended treatment. Artemether, or quinine, is an acceptable alternative if parenteral artesunate is not available: artemether 3.2 mg/kg BW IM given on admission then 1.6 mg/ kg BW per day*.</td>
<td>5 IU/kg 3.2 mg/kg</td>
</tr>
<tr>
<td>Artemotil injection</td>
<td>No USP monograph</td>
<td>Not on the 17th EML Ph. Int.: Available strengths: 50 mg/ml (paediatric formulation), 75 mg/ml, 150 mg/ml (adult formulation)</td>
<td>Artecef® 150 must only be applied via IM route. 3-day treatment course Initial dose: injection of 4.8 mg artemotil per kg BW evenly divided over both anterior thighs. Follow-up doses: 1.6 mg per kg BW after 6, 24, 48 and 72 hours in alternating thighs.</td>
<td>5 IU/kg 4.8 mg/kg</td>
</tr>
<tr>
<td>Ephedrine sulfate injection</td>
<td>Not more than 1.7 USP Endotoxin Units per mg of ephedrine sulfate</td>
<td>Not on the 17th EML Ph. Int.: Strength 50 mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ergometrine hydrogen maleate injection</td>
<td>No USP monograph</td>
<td>Ph. Int.: Ergometrine hydrogen maleate for parenteral use contains not more than 700.0 IU of endotoxin RS per mg Strength in EML: 200 μg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melarsoprol injection</td>
<td>No USP monograph</td>
<td>17th EML: Injection: 3.6% solution (180 mg in 5 ml ampoule)</td>
<td>Treatment of T. brucei rhodesiense and T. brucei gambiense with meningo encephalitic involvement (see above), by slow IV injection, ADULT and CHILD, dose gradually increased from 1.2 mg/kg to maximum of 3.6 mg/kg daily in courses of 3–4 days with intervals of 7–10 days between courses; alternatively for T. brucei gambiense infection, 2.2 mg/kg daily for 10 days.</td>
<td>5 IU/kg 3.6 mg/kg</td>
</tr>
<tr>
<td>Ph.Int. monographs lacking endotoxin limit</td>
<td>Endotoxin limits in respective USP monographs</td>
<td>Info on strength of dosage form (EML or PH.INT)</td>
<td>Info on dosage and route of application</td>
<td>K</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>----------------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>Magnesium sulfate injection</td>
<td>Not more than 0.09 USP Endotoxin Units per mg of magnesium sulfate (MgSO4·7H2O)</td>
<td>17th EML: 500 mg of magnesium sulfate hepta-hydrate per ml in 2 or 10 ml ampoule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxytocin injection</td>
<td>Not more than 35.7 Endotoxin Units per USP Oxytocin Unit</td>
<td>17th EML: 10 IU in 1 ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentamidine isetionate powder for injection</td>
<td>No monograph</td>
<td>17th EML: Powder for injection: 200 mg (as isetionate) in vial.</td>
<td>Visceral leishmaniasis (unresponsive to, or intolerant of, antimonial compounds), by deep IM injection or by IV infusion, ADULT and CHILD, 4 mg/kg 3 times a week for 5–25 weeks or longer, until 2 consecutive splenic aspirates 14 days apart are negative (see page 186)</td>
<td>5 IU/kg</td>
</tr>
<tr>
<td>Prednisolone sodium phosphate injection</td>
<td>Not more than 5.0 IU USP Endotoxin Units per mg prednisolone phosphate</td>
<td>Not in 17th EML. Strength in 12th EML: prednisolone powder for injection, 20 mg, 25 mg (as sodium phosphate or sodium succinate) in vial.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinine dihydrochloride injection</td>
<td>No monograph</td>
<td>17th EML: Injection: 300 mg quinine hydrochloride/ml in 2 ml ampoule.</td>
<td>Treatment of multidrug-resistant P. falciparum malaria (in patients unable to take quinine by mouth), by slow IV infusion, (over 4 hours) ADULT, initially 20 mg/kg quinine dihydrochloride followed by 10 mg/kg every 8 hours; CHILD, initially 20 mg/kg quinine dihydrochloride followed by 10 mg/kg every 12 hours; initial dose should be halved in patients who have received quinine, quinidine or mefloquine during the previous 12–24 hours (see page 198)</td>
<td>5 IU/kg</td>
</tr>
<tr>
<td>Zidovudine IV infusion</td>
<td>Not more than 1.0 IU of USP Endotoxin Units per mg of zidovudine</td>
<td>Strength in 17th EML: solution for IV infusion injection: 10 mg/ml in 20 ml vial</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
All expressions using “IU of endotoxin RS” should be changed to “IU of endotoxin” as it is sufficient to require that the WHO International Standard for endotoxin has to be used as a standard (or an endotoxin reference standard that has been calibrated against this standard, see 3.4 Test for bacterial endotoxins, section Preparation of standard endotoxin stock solution).

Niclosamidum
Niclosamide

Niclosamide, anhydrous
Niclosamide monohydrate

Draft revision of a monograph for inclusion in The International Pharmacopoeia (September 2013). Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidth@who.int. All working documents are posted for comment at http://www.who.int/medicines.

Note from the Secretariat. Niclosamide suffers pseudopolymorphic and polymorphic transformations when exposed to different conditions. Following investigations into these transitions by a WHO Collaborating Centre it is proposed to revise the monographs on Niclosamide and Niclosamide tablets. Deleted sections are indicated by […]

Molecular formula. \(C_{13}H_8C_12N_2O_4\) (anhydrous); \(C_{13}H_8C_12N_2O_4\cdot H_2O\) (monohydrate).

Relative molecular mass. 327.1 (anhydrous); 345.1 (monohydrate)

Graphic formula

![Graphic formula of Niclosamide](image)

\(n = 0\) (anhydrous)
\(n = 1\) (monohydrate)

Chemical name. 2’,5-Dichloro-4’-nitrosalicylanilide; 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide; CAS Reg. No. 50-65-7 (anhydrous).

2’,5-Dichloro-4’-nitrosalicylanilide monohydrate; 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide monohydrate; CAS Reg. No. 73360-56-2 (monohydrate).

[...]
Additional information. Anhydrous Niclosamide is hygroscopic. Niclosamide monohydrate may exhibit polymorphism.

[...]

Identity tests

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

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. For the anhydrous substance the infrared absorption spectrum is concordant with the spectrum obtained from niclosamide RS which has been dried at 100–105 °C for 4 h, or with the reference spectrum of niclosamide. For the monohydrate, dry the substance to be examined and the niclosamide RS at 100–105 °C for 4 h. The infrared absorption spectrum of the dried substance is concordant with the spectrum obtained from the dried niclosamide RS or with the reference spectrum of niclosamide. The infrared absorption spectrum is concordant with the reference spectrum of a relevant form of niclosamide.

[...]

Niclosamidi compressi
Niclosamide tablets

[...]

Identity tests
Heat a quantity of the powdered tablets equivalent to 0.5 g of Niclosamide with 25 ml of ethanol (~750 g/l) TS, filter while hot and evaporate to dryness on a water-bath. Dry the residue at 100–105 °C for 4 h.

The residue complies either with test A alone or with tests B, C and D.

A. Carry out the examination with the dried residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from niclosamide RS, which has been dried at 100–105 °C for 4 h, or with the reference spectrum of niclosamide.

[...]

Sulfamethoxazoli et trimethoprimi infusio intraveno
Sulfamethoxazole and trimethoprim intravenous infusion

Draft revision of a monograph for inclusion in The International Pharmacopoeia (July 2013). Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidth@who.int. All working documents are posted for comment at http://www.who.int/medicines.
**Category.** Antibacterials.

**REQUIREMENTS**

Comply with the monograph for Parenteral preparations.

**Definition.** Sulfamethoxazole and Trimethoprim intravenous infusion is a sterile solution containing Trimethoprim and sodium derivative of Sulfamethoxazole. It is prepared immediately before use by diluting Sulfamethoxazole and Trimethoprim sterile concentrate according to the manufacturers’ instructions.

**SULFAMETHOXAZOLE AND TRIMETHOPRIM STERILE CONCENTRATE**

**Description.** A colourless or slightly yellow solution.

**Storage:** Sulfamethoxazole and Trimethoprim sterile concentrate should be kept in tightly-closed, single-dose, light-resistant glass-containers.

**Additional information.** Strengths in the current WHO Model List of Essential Medicines: 80 mg per ml Sulfamethoxazole, 16 mg per ml Trimethoprim in 5 ml or 10 ml ampoule. Strengths in the current WHO Model List of Essential Medicines for Children: 80 mg per ml Sulfamethoxazole, 16 mg per ml Trimethoprim in 5 ml or 10 ml ampoule.

**REQUIREMENTS**

Comply with the monograph for Parenteral preparations.

**Definition.** Sulfamethoxazole and Trimethoprim sterile concentrate is a sterile solution containing Trimethoprim and the sodium derivative of Sulfamethoxazole. It contains not less than 90.0% and not more than 110.0% of the amounts of Sulfamethoxazole (C_{10}H_{11}N_{3}O_{3}S) and Trimethoprim (C_{14}H_{18}N_{4}O_{3}) stated on the label.

**Identity tests**

Either tests A or D together with E and F, or tests B and C may be applied.

A. Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 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 100 volumes of dichloromethane R, 10 volumes of methanol R and 5 volumes of dimethylformamide R as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions in methanol R. For solution (A) evaporate to dryness on a steam bath a volume of the concentrate, containing about 0.16 g of Sulfamethoxazole, shake the residue with 8 ml of methanol R and filter. For solution (B) use 20 mg of sulfamethoxazole RS and 4 mg of trimethoprim 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 spots obtained with solution (A) correspond in position, appearance and intensity to those obtained with solution (B).
A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and the conditions described above under test A.1. Spray the plate with potassium iodobismuthate TS2 solution.

The principal spots obtained with solution (A) correspond in position, appearance and intensity to those obtained with solution (B).

B. Add drop-wise to 75 ml of hydrochloric acid (~3.65 g/l) TS a volume of the concentrate containing about 0.8 g of Sulfamethoxazole, stirring continuously. Allow the suspension to stand for 5 minutes and filter through a sintered-glass filter. Wash the residue with 10 ml of water R, recrystallize from ethanol (~750 g/l) TS and dry at 105 °C. Dissolve the residue in a minimum volume of sodium carbonate (~50 g/l) TS, add hydrochloric acid (~36.5 g/l) TS drop-wise until precipitation is complete, filter, wash the residue sparingly with water R and dry at 105 °C. Carry out the test as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum of the residue is concordant with the spectrum obtained from sulfamethoxazole RS or with the reference spectrum of sulfamethoxazole.

C. To a volume of the concentrate containing about 80 mg of Trimethoprim add 30 ml of sodium hydroxide (~4 g/l) TS and extract with two quantities, each of 50 ml, of dichloromethane R. Wash the combined extracts with two quantities, each of 10 ml, of sodium hydroxide (~4 g/l) TS and then with 10 ml of water R. Shake with 5 g of anhydrous sodium sulfate R, filter and evaporate the filtrate to dryness. Carry out the test as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum of the residue is concordant with the spectrum obtained from trimethoprim RS or with the reference spectrum of trimethoprim.

D. See the test described under Assay method A. The retention times of two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

E. Dilute a volume of concentrate containing about 80 mg of sulfamethoxazole to 10 ml with water R. Add 1 ml of sodium hydroxide (~4 g/l) TS and 3 ml of 1% copper sulphate (~10g/l) TS drop by drop, until the colour change is complete. Green precipitates are produced.

F. Evaporate a volume of concentrate containing 32 mg of trimethoprim to dryness on a water bath. To the residue, add a drop of ammonium vanadate TS, a dark brown colour is produced.

**pH value (1.13).** pH of the solution, 9.5–11.0

**Bacterial endotoxins.** Carry out the test described under 3.4 Test for bacterial endotoxins, contains not more than 6 IU of endotoxin per mg Sulfamethoxazole.

Related substances

**Trimethoprim-related substances**

Carry out the test as described under 1.14.1. Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 97 volumes of chloroform R, 7.5 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS as the mobile
phase. Apply separately to the plate 10 µl of each of the following three solutions. For solution (A) transfer an accurately measured volume of the concentrate, containing about 48 mg of Trimethoprim, to a glass-stoppered, 50 ml centrifuge tube. Add 15 ml of hydrochloric acid (~2.19 g/l) TS and mix. Add 15 ml of dichloromethane R, shake for 30 seconds and centrifuge for 3 minutes. Transfer the supernatant layer to a 125 ml separator. Extract the dichloromethane layer in the centrifuge tube with 15 ml of hydrochloric acid (~2.19 g/l) TS, centrifuge and add the aqueous layer to the separator. Add 2 ml of sodium hydroxide (~100 g/l) TS to the solution in the separator and extract with three 20 ml portions of dichloromethane R, collecting the organic layer in a 125 ml conical flask. Evaporate the dichloromethane under a stream of nitrogen to dryness. Dissolve the residue in 1.0 ml of a mixture of equal volumes of dichloromethane R and methanol R (solvent mixture). For solution (B) use 48 mg of trimethoprim RS per ml solvent mixture. For solution (C) dilute an accurately measured volume of solution (B) with the solvent mixture to obtain a solution of 240 µg of trimethoprim RS per ml. After removing the plate from the chromatographic chamber allow it dry in air, spray with ferric chloride/potassium ferricyanide TS1 and examine the chromatogram in ultraviolet light (254 nm).

Trimethoprim and related substances have the following Rf values: trimethoprim about 0.5; and the trimethoprim degradation product about 0.6–0.7. In the chromatogram obtained with solution (A) any spot corresponding to the trimethoprim degradation product is not greater in size and intensity than the spot obtained with solution (C) (0.5%). Disregard any spots due to concentrate excipients at about Rf 0.1.

**Sulfamethoxazole related substances**

Either test A or test B may be applied.

A. Carry out the test as described under 1.14.1. Thin-layer chromatography, using silica gel R5 as the coating substance. As the mobile phase use a mixture of 25 volumes of ethanol/methanol (95/5) TS, 25 volumes of heptane R, 25 volumes of dichloromethane R and 7 volumes of glacial acetic acid R. Apply separately to the plate 10 µl of each of the following five solutions. For solution (A) transfer an accurately measured volume of the concentrate, containing about 160 mg of Sulfamethoxazole, to an evaporating dish. Evaporate the sample to dryness using a steam bath. Reconstitute the residue with 16 ml of ammonia/ethanol/methanol (1/95/5) TS. For solution (B) use 10 mg of sulfamethoxazole RS per ml ammonia/ethanol/methanol (1/95/5) TS. For solution (C) use 0.05 mg of sulfanilamide R per ml ammonia/ethanol/methanol (1/95/5) TS. For solution (D) use 0.03 mg of sulfanilic acid R per ml ammonia/ethanol/methanol (1/95/5) TS. For solution (E) dissolve 10 mg of sulfamethoxazole RS in 1.0 ml of a solution containing 0.05 mg of sulfanilamide R and 0.03 mg of sulfanilic acid R per ml of ammonia/ethanol/methanol (1/95/5) TS. After removing the plate from the chromatographic chamber allow it dry in air, spray with 4-dimethylaminobenzaldehyde TS7, allow the plate to stand for 15 minutes and examine the chromatogram.

In the chromatogram obtained with solution (A) any spot corresponding to sulfanilamide is not greater in size or intensity than the spot obtained with solution (C) (0.5%) and any spot corresponding to sulfanilic acid is not greater in size or intensity than the spot obtained with solution (D) (0.3%). The test is not valid unless the chromatogram obtained with solution (E) shows three clearly separated principal spots.
B. Carry out the test described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay method A.

Prepare the following solutions. For solution (1) transfer 1.0 ml of the concentrate containing 80 mg of Sulfamethoxazole into a test tube. Add 7 ml of the mobile phase and mix. Transfer 5.0 ml of this solution to a 100 ml volumetric flask, dilute with the mobile phase to volume, mix and filter. For solution (2) prepare 5 mg/ml of sulfanilamide R in ammonia/methanol (10/90) TS. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase. For solution (3) prepare 3 mg/ml of sulfanilic acid R in ammonia/methanol (10/90) TS. Dilute 5.0 ml of this solution to 50.0 ml with mobile phase. For solution (4) transfer 1.0 ml of each of solution (2) and (3) into a 200 ml volumetric flask and make up to volume with mobile phase. For solution (5) accurately weigh 50 mg of sulfamethoxazole RS in a 100 ml volumetric flask and dilute with solution (4) to volume.

Inject separately 20 µl each of solutions (1), (4) and (5) and record the chromatogram for 1.5 times the retention time of sulfamethoxazole.

In the chromatogram obtained with solution (5) the three principal peaks are eluted at the following relative retention times with reference to sulfamethoxazole (retention time about 11 minutes): sulfanilic acid about 0.2; sulfanilamide about 0.3. The test is not valid unless for solution (5) the resolution factor between the peaks due to sulfanilic acid and to sulfanilamide is at least 5.0 and the resolution factor between the peaks due to sulfanilamide and sulfamethoxazole is at least 10.

Measure the areas of the peak responses obtained in the chromatograms from solution (1) and (4). In the chromatogram obtained with solution (1) the area of any peak corresponding to sulfanilic acid is not more than the area of the peak due to sulfanilic acid in the chromatogram obtained with solution (4) (0.3%), and the area of any peak corresponding to sulfanilamide is not greater than the area of the peak due to sulfanilamide in the chromatogram obtained with solution (4) (0.5%).

Assay

Either method A or methods B and C may be applied.

A. 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 particles of base-deactivated silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm). (Hypersil BDS C18 has been found suitable). As the mobile phase use a solution prepared as follows: mix 1400 ml of water R, 400 ml of acetonitrile R and 2.0 ml of triethylamine R in a 2000 ml volumetric flask. Allow to equilibrate to room temperature and adjust with acetic acid (~10 g/l) TS to pH 5.9. Dilute to volume with water R and filter.

Prepare the following solutions. For solution (1) transfer an accurately measured volume of the concentrate containing about 80 mg of Sulfamethoxazole into a 50 ml volumetric flask. Add methanol R to volume and mix. Transfer 5.0 ml of this solution to a 50 ml volumetric flask, dilute with the mobile phase to volume, mix and filter. For solution (2) prepare a solution of 0.32 mg of trimethoprim RS and 1.60 mg of sulfamethoxazole RS per ml methanol R. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.
Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject separately 20 µl each of solutions (1) and (2) and record the chromatogram for 1.5 times the retention time of sulfamethoxazole. In the chromatogram obtained with solution (2) the two principal peaks elute in the order: Trimethoprim (retention time about 6 minutes); Sulfamethoxazole (retention time about 11 minutes). The test is not valid unless the resolution factor between the peaks due to sulfamethoxazole and to trimethoprim is at least 5.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of Sulfamethoxazole \((\text{C}_{10}\text{H}_{11}\text{N}_{3}\text{O}_{3}\text{S})\) and Trimethoprim \((\text{C}_{14}\text{H}_{18}\text{N}_{4}\text{O}_{3})\) in the concentrate, using the declared content of \(\text{C}_{10}\text{H}_{11}\text{N}_{3}\text{O}_{3}\text{S}\) and \(\text{C}_{14}\text{H}_{18}\text{N}_{4}\text{O}_{3}\) in sulfamethoxazole RS and trimethoprim RS.

B. To an accurately measured volume of the concentrate, containing about 48 mg of Trimethoprim, add 30 ml of sodium hydroxide \((-4 \text{ g/l})\) TS and extract with four quantities, each of 50 ml, of dichloromethane R, washing each extract twice with a quantity of 10 ml of sodium hydroxide \((-4 \text{ g/l})\) TS. Combine the dichloromethane extracts and extract with four quantities of 50 ml of acetic acid \((-60 \text{ g/l})\) TS. Wash the combined aqueous extracts with 5 ml of dichloromethane R and dilute to 250.0 ml with acetic acid \((-60 \text{ g/l})\). To 10.0 ml of this solution add 10 ml of acetic acid \((-60 \text{ g/l})\) and dilute to 100.0 ml with water R. Measure the absorbance of the resulting solution at the maximum at 271 nm.

Calculate the amount of Trimethoprim \((\text{C}_{14}\text{H}_{18}\text{N}_{4}\text{O}_{3})\) using the absorptivity value of 20.4

\[
A^{\%}_{1\text{cm}} = 20.4
\]

C. To an accurately measured volume of the concentrate containing about 0.4 g of Sulfamethoxazole add 60 ml of water R and 10 ml of hydrochloric acid \((-420 \text{ g/l})\) TS. Add 3 g of potassium bromide R, cool in ice and titrate slowly with sodium nitrite \((0.1 \text{ mol/l})\) VS, stirring constantly and determining the end-point potentiometrically.

Each ml of sodium nitrite \((0.1 \text{ mol/l})\) VS is equivalent to 25.33 mg of Sulfamethoxazole \((\text{C}_{10}\text{H}_{11}\text{N}_{3}\text{O}_{3}\text{S})\).

New reagents needed to be added to Ph.Int.:

Ethanol/methanol (95/5) TS
Procedure. To 5 ml of methanol R add 95 ml of dehydrated ethanol R.

Ammonia/ethanol/methanol (1/95/5) TS
Procedure. To 1 ml of ammonia \((-206 \text{ g/l})\) TS add 99 ml of Ethanol / methanol (95/5) TS.

Ammonia/methanol (10/90) TS
Procedure. To 10 ml of ammonia \((-206 \text{ g/l})\) TS add 90 ml of methanol R.

Acetic acid \((-10 \text{ g/l})\) TS
Acetic acid \((-300 \text{ g/l})\) TS, diluted with water to contain about 10 g of C2H4O per litre.
Hydrochloric acid (~3.65 g/l) TS
Hydrochloric acid (~250 g/l) TS, dilute with water to contain 3.65 g of HCl in 1000 ml.

Hydrochloric acid (~2.19 g/l) TS
Hydrochloric acid (~250 g/l) TS, dilute with water to contain 2.19 g of HCl in 1000 ml.

Hydrochloric acid (~36.5 g/l) TS
Hydrochloric acid (~250 g/l) TS, dilute with water to contain 36.5 g of HCl in 1000 ml.

Sodium hydroxide (~4 g/l) TS
A solution of sodium hydroxide R containing about 4 g/l of NaOH (approximately 0.1 mol/l).

Sodium hydroxide (~100 g/l) TS
A solution of sodium hydroxide R containing about 100 g/l of NaOH (approximately 2.5 mol/l).

Ferric chloride/potassium ferricyanide TS1
Procedure. Dissolve 2 g of ferric chloride R and 0.5 g of potassium ferricyanide R in sufficient water to produce 20 ml.

Note. Ferric chloride/potassium ferricyanide TS2 must be freshly prepared.

4-Dimethylaminobenzaldehyde TS7
Dissolve 0.1 g of 4-dimethylaminobenzaldehyde R in 1 ml of hydrochloric acid (~420 g/l) TS, dilute with ethanol (~750 g/l) to produce 100 ml.

Ammonium vanadate TS
Dissolve 0.5 g of Ammonium vanadate in 1.5 ml water and dilute to 100 ml with sulfuric acid.

Copper(II) Sulfate (10 g/L) TS
A solution of Copper(II) sulfate R containing 10 g of CuSO4 per litre.

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Medroxyprogesterone acetate

Draft revision of a monograph for inclusion in The International Pharmacopoeia (September 2013). Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidt@who.int. All working documents are posted for comment at http://www.who.int/medicines.
C\textsubscript{24}H\textsubscript{34}O\textsubscript{4}

**Relative molecular mass.** 386.5

**Chemical name.** 17-Hydroxy-6α-methylpregn-4-ene-3,20-dione acetate; 17-(acetyloxy)-6α-methylpregn-4-ene-3,20-dione; CAS Reg. No. 71-58-9.

**Description.** A white or almost white, crystalline powder; odourless or almost odourless.

**Solubility.** Practically insoluble in water; soluble in acetone R and dioxan R; slightly soluble in ethanol (~750 g/l) TS, methanol R and ether R.

**Category.** Progestogen.

**Storage.** Medroxyprogesterone acetate should be kept in a tight container, protected from light.

**Requirements**

**Definition.** Medroxyprogesterone acetate contains not less than 97.0% and not more than the equivalent of 103.0% of C\textsubscript{24}H\textsubscript{34}O\textsubscript{4}, calculated with reference to the dried substance.

**Identity tests**

Either tests A and C or test 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 medroxyprogesterone acetate RS or with the reference spectrum of medroxyprogesterone acetate.

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica R5 as the coating substance and a mixture of 10 volumes of dichloromethane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 10 μl of each of the following three solutions in dichloromethane R. For solution (A) use 5 mg of Medroxyprogesterone acetate per ml. For solution (B) use 5 mg of medroxyprogesterone acetate RS per ml. For solution (C) use 5 mg of medroxyprogesterone acetate RS and 0.2 mg of medroxyprogesterone acetate impurity F RS per ml. After removing the plate from the chromatographic chamber, heat it at 120 °C for 30 minutes, spray with 4-toluenesulfonic acid/ethanol TS and heat further at 120 °C for 10 minutes. Allow the plate to cool and examine the chromatogram in ultraviolet light (365 nm). The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).
B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described under test B.1, but spray the plate with a mixture of equal volumes of sulfuric acid R and ethanol (~750 g/l) TS and heat further at 120 °C for 10 minutes. Allow the plate to cool and examine the chromatogram in daylight. The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots.

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

C. Use 20 mg; it yields the reaction described under 2.1 General identification tests as characteristic of acetylated substances.

**Specific optical rotation.** Use a 10 mg/ml solution in acetone R; $[\alpha]^{20}_{\text{D}} = +47°$ to $+53°$.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 105 °C for 3 hours; it loses not more than 10 mg/g.

**Impurity F**

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 a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3 µm). (Welch Ultimate XB-C18 has been found suitable).

As the mobile phase, use a solution prepared as follows: mix 44 volumes of water R and 56 volumes of acetonitrile R.

For solution (1) dissolve 20 mg of Medroxyprogesterone acetate in 5.0 ml of acetonitrile R and dilute to 10.0 ml with water R. For solution (2) use 0.01 mg of medroxyprogesterone acetate impurity F RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 200 nm.

Inject 25 µl of solution (1) and (2). In the chromatogram obtained with solution (2), impurity F is eluted at a relative retention of about 1.8 with reference to medroxyprogesterone acetate (retention time about 8 minutes).

In the chromatogram obtained with solution (1), the area of any peak corresponding to impurity F is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

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 tetrahydrofuran R, 45 volumes of tert-butyl methyl ether R and 45 volumes of heptane R as the mobile phase.
Apply separately to the plate 10 l of each of the following two solutions in dichloromethane R. For solution (A) use 20 mg of Medroxyprogesterone acetate per ml. For solution (B) use 20 mg of medroxyprogesterone acetate RS and 0.1 mg of medroxyprogesterone acetate impurity F RS per ml.

Develop the plate for a distance of about 10 cm. Allow it to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase. After removing the plate from the chromatographic chamber, heat it at 100 °C to 105 °C for 30 minutes and spray with 4-toluenesulfonic acid/ethanol TS. Heat again at 120 °C for 10 minutes, allow to cool and examine the chromatogram in ultraviolet light (365 nm).

In the chromatogram obtained with solution (B) impurity F has a Rf value of about 0.78 and medroxyprogesterone acetate a Rf value of about 0.70. The test is not valid unless the chromatogram obtained with solution (B) shows two clearly separated spots. In the chromatogram obtained with solution (A) any spot due to impurity F is not more intense than the corresponding spot in the chromatogram obtained with solution (B) (0.5%).

**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 a stainless steel column (25 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). (Altima C18 has been found suitable.)

Maintain the column temperature at 45 °C.

As the mobile phase, use a solution prepared as follows: mix 15 volumes of tetrahydrofuran R, 23 volumes of acetonitrile R and 65 volumes of water R and filter.

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

For solution (1) dissolve 20 mg of Medroxyprogesterone acetate in the dissolution solvent and dilute to 10.0 ml with the solvent mixture. For solution (2) dilute 1.0 ml of solution (1) to 100.0 ml with the solvent mixture. For solution (3) dilute 1.0 ml of solution (2) to 10.0 ml with the solvent mixture. For solution (4) use 2 mg of medroxyprogesterone acetate RS and 0.01 mg of medroxyprogesterone acetate impurity G RS per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject separately 20 µl of solution (1), (2), (3) and (4). Record the chromatogram for about twice the retention time of medroxyprogesterone acetate in solution (2).

The following peaks are eluted at the following relative retention with reference to the peak of medroxyprogesterone acetate (retention time about 27 minutes): impurity A about 0.3; impurity I about 0.5; impurity H about 0.65; impurity B about 0.7; impurity C about 0.8; impurity G about 0.85; impurity D about 0.9; impurity E about 0.95. The test is not valid unless in the chromatogram obtained with solution (4) the resolution factor between the peaks due to impurity G and due to medroxyprogesterone acetate is at least 3.3.
In the chromatogram obtained with solution (1):

- The area of any peak corresponding to impurity D is not greater than the area of the principal peak obtained with solution (2) (1.0%);

- The area of any peak corresponding to impurity B is not greater than 0.7 times the area of the principal peak obtained with solution (2) (0.7%);

- The area of any peak corresponding to impurity A, when multiplied by a correction factor of 1.5, is not greater than 3 times the area of the principal peak obtained with solution (3) (0.3%);

- The area of any peak corresponding to impurity G, when multiplied by a correction factor of 2.6, is not greater than 2 times the area of the principal peak obtained with solution (3) (0.2%);

- The area of any peak corresponding to impurity C, E or I is not greater than 2 times the area of the principal peak obtained with solution (3) (0.2%);

- The area of any other impurity peak is not greater than the area of the principal peak obtained with solution (3) (0.1%);

- The sum of the areas (corrected, where necessary) of all the peaks, other than the principal peak, is not greater than 1.5 times the area of the principal peak obtained with solution (2) (1.5%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (3) (0.05%).

**Assay**

Dissolve about 0.1 g, accurately weighed, in ethanol (~750g/l) TS to produce 100 ml; dilute 1.0 ml of this solution to 100 ml with the same solvent.

Measure the absorbance of the diluted solution in a 1 cm layer at the maximum at about 241 nm and calculate the content of C_{24}H_{34}O_{4} using the absorptivity value of 42.6 \( A_{\text{1cm}}^{\%} = 426 \) \( A_{\text{1cm}}^{\%} = 426 \)

**Impurities**

A. R1=OH, R2= CH3, R3=CO-CH3; 6-hydroxy-6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-hydroxymedroxyprogesterone acetate),
B. R1=R2=H, R2=CH3; 17-hydroxy-6-methylpregn-4-ene-3,20-dione (medroxyprogesterone),

C. 6,17a-dimethyl-3,17-dioxo-D-homoandrost-4-en-17a-yl acetate,

D. R1=CH3, R2=H, R3=CO-CH3; 6-methyl-3,20-dioxopregn-4-en-17-yl acetate (6-epimedroxyprogesterone acetate),

E. R1+R2=CH2, R3=CO-CH3; 6-methylidene-3,20-dioxopreg-4-en-17-yl acetate (6-methylenehydroxyprogesterone acetate),

F. 6-methyl-3,20-dioxo-5-pregn-17-yl acetate (4,5-dihydromedroxyprogesterone acetate),

G. 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate (megestrol acetate),
H. R1=R2=H, R3=CO-CH3; 3,20-dioxopregn-4-en-17-yl acetate (hydroxyprogesterone acetate)

I. (17ab)-17a-hydroxy-6,17a-dimethyl-D-homoandrost-4-ene-3,17-dione.

Reference substances to be established
medroxyprogesterone acetate RS
medroxyprogesterone acetate impurity F RS
medroxyprogesterone acetate impurity G RS

Fluconazoli
Fluconazole

Draft revision of a monograph for inclusion in The International Pharmacopoeia (July 2013). Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidt@who.int. All working documents are posted for comment at http://www.who.int/medicines.

C_{13}H_{12}F_{2}N_{6}O

Relative molecular mass. 306.3

Chemical name. 2-(2,4-Difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol; CAS Reg. No.86386-73-4.
Description. A white or almost white, crystalline powder.

Solubility. Slightly soluble in water, freely soluble in methanol, soluble in acetone.

Category. Antifungal.

Storage. Fluconazole should be kept in a tightly closed container, stored below 30 °C.

Additional information. Fluconazole is hygroscopic and exhibits polymorphism.

Requirements

Definition. Fluconazole contains not less than 99.0% and not more than 101.0% of C_{13}H_{12}F_{2}N_{6}O, calculated with reference to the dried substance.

Identity tests

Either test A alone 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 fluconazole RS or with the reference spectrum of fluconazole. If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and fluconazole RS in a small amount of dichloromethane R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from fluconazole RS.

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 80 volumes of dichloromethane R, 20 volumes of methanol R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 μl of each of the following three solutions in methanol R. For solution (A) use 10 mg of Fluconazole per ml. For solution (B) use 10 mg of fluconazole RS per ml. For solution (C) use a solution containing 10 mg of fluconazole RS per ml and 1 mg of fluconazole impurity C RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air and 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). The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots.

C. The absorption spectrum (1.6) of a 200 μg/ml solution in ethanol R, when observed between 230 nm and 300 nm, exhibits maxima at about 261 nm and 267 nm and a minimum at about 264 nm; the ratio of the absorbance of a 1 cm layer at the maximum at about 261 nm to that at the minimum at about 264 is about 1.4.

Clarity and colour of solution. A solution of 1.0 g in 20 ml of methanol R is clear and colourless.

Heavy metals

Either test A or test B may be applied.
A. Use 1.0 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.

B. Use 1.0 g for the preparation of the test solution in methanol as described under 2.2.3 Limit test for heavy metals, Procedure 2; 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, using Method B and a platinum crucible.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5 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 particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm) (Capcell Pak® C18 MGII (4.6 x 250 mm, 5µm) has been found suitable.) As the mobile phase, use a mixture of 86 volumes of a (0.63 g/l) solution of ammonium formate R and 14 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase. For solution (1) use 10 mg of Fluconazole per ml. For solution (2) dilute 5 ml of solution (1) to 100 ml, then dilute 1 ml of this solution to 10 ml. For solution (3) use 0.1 mg of fluconazole impurity C RS per ml. For solution (4), transfer 1.0 ml of solution (3) to a 10 ml volumetric flask, add 1.0 ml of solution (1) and make up to volume.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 260 nm.

Inject separately 20 μl each of solutions (1), (2), (3) and (4). Record the chromatograms for about 3.5 times the retention time of fluconazole.

The peaks are eluted at the following relative retentions with reference to fluconazole (retention time about 11 minutes): impurity B about 0.4; impurity A about 0.5; impurity C about 0.8.

The test is not valid, unless in the chromatogram obtained with solution (4) the resolution between the peaks due to impurity C and fluconazole is at least 3.0.

In the chromatogram obtained with solution (1),

• The area of any peak corresponding to impurity A is not is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4 %),

• The area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.5 is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3 %),

• The area of any peak corresponding to impurity C is not is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1 %),
- The area of any other peak, other than the principal peak, is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1 %),

- The sum of the corrected area of any peak corresponding to impurity B and the areas of all other peaks, other than the principal peak, is not greater than 1.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6 %). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05 %).

**Assay**

Dissolve about 0.1 g, accurately weighed, in 50 ml of anhydrous acetic acid R and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 15.32 mg of C_{13}H_{12}F_{2}N_{6}O.

**Impurities**

A. (2RS)-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-3-(4H-1,2,4-triazol-4-yl)propan-2-ol,

B. 2-[2-fluoro-4-(1H-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol,

C. 1,1’-(1,3-phenylene)di-1H-1,2,4-triazole,
D. 2-(4-fluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol,

E. 1-[(6RS)-4,6-difluoro-6-(1H-1,2,4-triazol-1-yl)cyclohexa-1,4-dienyl]ethanone,

F. R=OH: (2RS)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl) propan-2-diol,
H. R=Br: (2RS)-1-bromo-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl) propan-2-ol,

G. [3-[[6RS]-2-(2,4-difluorophenyl)oxiran-2-yl[methyl]1H-1,2,4-triazol-1-yl]] methanesulfonic acid,
I. 4-amino-1-[(2RS)-2-(2,4-difluorophenyl)-2-hydroxy-3(1H-1,2,4-triazol-1-yl)propyl]-4H-1,2,4-triazolium.

[Note from Secretariat: chemical names and structures to be confirmed.]

Reference substances to be established:

Fluconazole RS
Fluconazole impurity C RS

Fluconazoli capsulae
Fluconazole capsules

Draft revision of a monograph for inclusion in The International Pharmacopoeia (July 2013). Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidth@who.int. All working documents are posted for comment at http://www.who.int/medicines.

Category. Antifungal.

Storage. Fluconazole capsules should be kept in a tightly closed container and stored at a temperature not exceeding 30 °C.


Requirements

Comply with the monograph for «Capsules».

Definition. Fluconazole capsules contain Fluconazole. They contain not less than 90.0% and not more than 110.0% of the amount of fluconazole ($C_{13}H_{12}F_2N_6O$) stated on the label.

Identity tests

Either tests A and C or tests B and C may be applied.
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 80 volumes of dichloromethane R, 20 volumes of methanol R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 μl of each of the following three solutions in methanol R. For solution (A) shake a quantity of the mixed contents of the capsules, containing about 100 mg of Fluconazole, with 10 ml of methanol R, filter, and use the clear filtrate. For solution (B) use 10 mg of fluconazole RS per ml. For solution (C) use a mixture of 10 mg of fluconazole RS and 10 mg of fluconazole impurity C RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and 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). The test is not valid, unless the chromatogram obtained with solution (C) shows two clearly separated spots.

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay method B. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. To a quantity of the capsule content, containing 2 mg of Fluconazole, add 10 ml of ethanol R, shake and filter. The absorption spectrum (1.6) of the resulting solution, when observed between 230 nm and 300 nm, exhibits maxima at 261 nm and 267 nm and a minimum at about 264 nm. The ratio of the absorbance of a 1 cm layer at the maximum at about 261 nm to that at the minimum at about 264 is about 1.4.

Related substances
Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay method B. Prepare the following solutions in the mobile phase. For solution (1) use an amount of the mixed contents of 20 capsules to produce a solution containing 10 mg of Fluconazole per ml and filter the solution. For solution (2) dilute 5 volumes of solution (1) to 100 volumes, then dilute 1 volume of this solution to 10 volumes. For solution (3) use 0.1 mg of fluconazole impurity C RS per ml. For solution (4), transfer 1.0 ml of solution (3) to a 10 ml volumetric flask, add 1.0 ml of solution (1) and make up to volume.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 260 nm.

Inject separately 20 μl each of solutions (1), (2), (3) and (4). Record the chromatograms for about 3.5 times the retention time of fluconazole.

The peaks are eluted at the following relative retentions with reference to fluconazole (retention time about 11 minutes): impurity B about 0.4; impurity A about 0.5; impurity C about 0.8.

The test is not valid, unless in the chromatogram obtained with solution (4), the resolution between the peaks due to impurity C and to fluconazole is at least 3.0

In the chromatogram obtained with solution (1),
The area of any peak corresponding to impurity A is not greater than 0.8 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

The area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.5 is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

The area of any peak corresponding to impurity C is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

The area of any other impurity peak, other than the principle peak, is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%),

The sum of the corrected area of any peak corresponding to impurity B and the areas of all peaks, other than the peak due to fluconazole, is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Dissolution test**

Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 500 ml of hydrochloric acid (~4 g/l) TS, rotating the basket at 100 revolutions per minute. At 45 minutes withdraw a sample of about 10 ml of the medium through a suitable 0.45 μm filter. Measure the absorbance (1.6) of a 1 cm layer of the filtered solution, suitably diluted if necessary, at the maximum at 261 nm. At the same time measure the absorbance (1.6) at the maximum at 261 nm of a solution containing 0.1 mg of fluconazole RS per ml in the dissolution medium, using the same solution as the blank.

For each of the capsules tested, calculate the total amount of fluconazole \((C_{13}H_{12}F_{2}N_{6}O)\) in the medium from the absorbances obtained and from the declared content of \(C_{13}H_{12}F_{2}N_{6}O\) in fluconazole RS. Use the requirements as described under 5.5 Dissolution test for solid oral dosage forms, Acceptance criteria to evaluate the results: The amount in solution is not less than 75% (Q) of the amount declared on the label.

**Assay**

Either test A or B may be applied.

A. Mix the contents of 20 capsules and transfer a quantity containing about 50 mg of Fluconazole, accurately weighed, to a 10 ml volumetric flask, and dilute to volume with hydrochloric acid (~4 g/l) TS. Shake to dissolve, filter a portion of this solution and dilute 10 ml of the filtered solution to 25 ml with the same solution. Measure the absorbance of a 1 cm layer at the maximum at about 261 nm.

At the same time measure the absorbance of a solution of 0.2 mg of fluconazole RS per ml of hydrochloric acid (~4 g/l) TS, prepared and examined in the same manner, and calculate the percentage content of fluconazole \((C_{13}H_{12}F_{2}N_{6}O)\) in the capsules, using the declared content of \(C_{13}H_{12}F_{2}N_{6}O\) in fluconazole RS.
B. 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 particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5μm). (Capcell Pak® C18 MGII (4.6x250 mm, 5μm) has been found suitable.) As the mobile phase, use a mixture of 86 volumes of a (0.63 g/l) solution of ammonium formate R and 14 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase. For solution (1) use an amount of the mixed contents of 20 capsules to produce a solution containing 0.5 mg of Fluconazole per ml and filter the solution. For solution (2) use 0.5 mg of fluconazole RS per ml. For solution (3) use a solution containing 0.01 mg of fluconazole impurity C RS per ml and 1 mg of fluconazole RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 260 nm.

Inject separately 20 μl of each of solutions (1), (2) and (3). The test is not valid, unless in the chromatogram obtained with solution (3), the resolution between the peaks due to impurity C and to fluconazole is at least 3.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of fluconazole (C₁₃H₁₂F₂N₆O) in the capsules, using the declared content of C₁₃H₁₂F₂N₆O in fluconazole RS.

Fluconazoli injectio
Fluconazole injection

Draft revision of a monograph for inclusion in The International Pharmacopoeia (July 2013). Please address any comments to Technologies, Standards and Norms, Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland. Or e-mail to schmidth@who.int. All working documents are posted for comment at http://www.who.int/medicines.

Description. A clear, colourless solution.

Category. Antifungal.

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

Additional information. Strength in the current WHO Model List of Essential Medicines: 2 mg/ml in vial.

Requirements

Complies with the monograph for «Parenteral preparations».
**Definition.** Fluconazole injection is a sterile solution of Fluconazole in Water for injections.

The solution is sterilized by a suitable method (see 5.8 Methods of sterilization). Fluconazole injection contains not less than 90.0% and not more than 110.0% of the amount of fluconazole \(\text{C}_{13}\text{H}_{12}\text{F}_{2}\text{N}_{6}\text{O}\) stated on the label.

**Identity tests**

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

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 80 volumes of dichloromethane R, 20 volumes of methanol R, and 1 volume of ammonia (~260 g/l) TS solution as the mobile phase. Apply separately to the plate 20 μl of each of the following three solutions. For solution (A) use the injection to be examined. For solution (B) use 2 mg of fluconazole RS per ml in methanol R. For solution (C) use a mixture of 2 mg of fluconazole RS per ml and 2 mg of fluconazole impurity C RS per ml in methanol R. After application allow the spots to dry in a current of air. Develop the plate. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and 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). The test is not valid, unless the chromatogram obtained with solution (C) shows two clearly separated spots.

B. 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 that of the principal peak in the chromatogram obtained with solution (2).

C. Dilute a volume of the injection containing 2 mg of Fluconazole to 10 ml with water R. The absorption spectrum (1.6) of the resulting solution, when observed between 230 nm and 300 nm, exhibits maxima at 261 nm and 267 nm, and a minimum at about 264 nm. The ratio of the absorbance of a 1 cm layer at the maximum at about 261 nm to that at the minimum at about 264 is about 1.4.

**pH value (1.3).** pH of the injection, 4.0–6.0.

**Related substances**

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given below under Assay. Prepare the following solutions in the mobile phase. For solution (1) use the injection to be examined. For solution (2) dilute 5 volumes of solution (1) to 100 volumes, then dilute 1 volume of this solution to 10 volumes. For solution (3) use 0.02 mg of fluconazole impurity C RS per ml. For solution (4) mix 1 volume of solution (3) with 1 volume of solution (1).

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 260 nm.
Inject separately 20 µl each of solutions (1), (2), (3) and (4). Record the chromato-
grams for about 3.5 times the retention time of fluconazole.

The peaks are eluted at the following relative retentions with reference to fluconazole
(retention time about 11 minutes): impurity B about 0.4; impurity A about 0.5; impurity
C about 0.8.

The test is not valid unless, in the chromatogram obtained with solution (4), the
resolution between the peaks due to impurity C and to fluconazole is at least 3.0

In the chromatogram obtained with solution (1),

- The area of any peak corresponding to impurity A is not greater than 0.8 times the
  area of the principal peak in the chromatogram obtained with solution (2) (0.4%),

- The area of any peak corresponding to impurity B, when multiplied by a correction
  factor of 1.5 is not greater than 0.3 times the area of the principal peak in the chroma-
togram obtained with solution (3) (0.3%);

- The area of any peak corresponding to impurity C is not greater than 0.1 times the
  area of the principal peak in the chromatogram obtained with solution (3) (0.1%);

- The area of any other impurity peak, other than the principal peak, is not greater than
  0.4 times the area of the principal peak in the chromatogram obtained with solution (2)
  (0.2%),

- The sum of the corrected area of any peak corresponding to impurity B and the
  areas of all peaks, other than the peak due to fluconazole, is not greater than 2 times
  the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %).

Disregard any peak with an area less than 0.1 times the area of the principal peak in
the chromatogram obtained with solution (2) (0.05%).

Assay

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 particles of silica gel,
the surface of which has been modified with chemically-bonded octadecylsilyl groups
(5µm). (Capcell Pak® C18 MGII (4.6x250 mm, 5µm) has been found suitable.)

As the mobile phase, use a mixture of 86 volumes of a (0.63 g/l) solution of
ammonium formate R and 14 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase. For solution (1) dilute 5.0 ml of
the injection to be examined to 20.0 ml. For solution (2) use 0.5 mg of fluconazole RS
per ml. For solution (3) use a solution containing 0.01 mg of fluconazole impurity C RS
per ml and 1 mg of fluconazole RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet
spectrophotometer set at a wavelength of 260 nm.

Inject separately 20 µl of each of solutions (1), (2) and (3). The test is not valid, unless
in the chromatogram obtained with solution (3), the resolution between the peaks due
to impurity C and due to fluconazole is at least 3.0.
Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of fluconazole (C\textsubscript{13}H\textsubscript{12}F\textsubscript{2}N\textsubscript{6}O) in the injection, using the declared content of C\textsubscript{13}H\textsubscript{12}F\textsubscript{2}N\textsubscript{6}O in fluconazole RS.

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins,; contains not more than 0.416 IU of endotoxin RS per mg of Fluconazole.