SULFAMETHOXAZOLE AND TRIMETHOPRIM ORAL SUSPENSION

Draft proposal for The International Pharmacopoeia
(April 2012)

DRAFT FOR COMMENT

This document was provided by a quality control expert. Should you have any comments thereon, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mails: kopp@who.int with a copy to Ms C. Mendy mendyc@who.int by 19 June 2012.

In order to speed up the process for receiving draft monographs and for sending comments, please let us have your e-mail address (to bonnyw@who.int) and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.
## SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/12.472

*Draft proposal for The International Pharmacopoeia: Sulfamethoxazole and Trimethoprim oral suspension*

<table>
<thead>
<tr>
<th>Activity</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>First draft received from collaborating laboratory</td>
<td>March 2012</td>
</tr>
<tr>
<td>Draft monograph sent out for comment</td>
<td>May 2012</td>
</tr>
<tr>
<td>Discussion at consultation on specifications for medicines and quality control laboratory issues</td>
<td>May 2012</td>
</tr>
<tr>
<td>Consolidation of comments</td>
<td>June - July 2012</td>
</tr>
<tr>
<td>Revision of draft monograph as per comments received</td>
<td>July-August 2012</td>
</tr>
<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for adoption</td>
<td>October 2012</td>
</tr>
<tr>
<td>Further follow-up action as required</td>
<td></td>
</tr>
</tbody>
</table>
Draft proposal for *The International Pharmacopoeia*

*SULFAMETHOXAZOLI ET TRIMETHOPRIMI SOLUTIONUM PERORALUM*

*SULFAMETHOXAZOLE AND TRIMETHOPRIM ORAL SUSPENSION*

**Category.** Antibacterials.

**Storage.** The oral suspension should be kept in a tightly closed container, protected from light.

**Additional information.** Strength in the current WHO Model list of essential medicines: 200 mg Sulfamethoxazole, 40 mg Trimethoprim per 5 ml. Strength in the current WHO Model list of essential medicines for children: 200 mg Sulfamethoxazole, 40 mg Trimethoprim per 5 ml.

**Requirements**

Complies with the monograph for “Liquid preparations for oral use”.

**Definition.** Sulfamethoxazole and Trimethoprim oral suspension is a suspension containing Sulfamethoxazole and Trimethoprim in a suitable vehicle which may be flavoured. The oral suspension contains not less than 90.0% and not more than 110.0% of Sulfamethoxazole (C₁₀H₁₁N₃O₃S) and Trimethoprim (C₁₄H₁₈N₄O₃) stated on the label.

**Identity tests**

- Either test A or B may be applied. Test C may be applied for identification of Trimethoprim.

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. For solution (A), add 20 ml of methanol R to 5 ml of the oral suspension, mix, shake with 10 g of anhydrous sodium sulfate R, centrifuge and use the supernatant liquid. For solution (B) use 20 mg of sulfamethoxazole RS and 4 mg of trimethoprim RS per ml methanol R. 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 solutions 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 dilute potassium iodobismuthate solution TS2.

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

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

C. To a quantity of the oral solution containing 50 mg of Trimethoprim add 30 ml of sodium hydroxide (~ 4 g/l) TS and extract with two quantities of 50 ml of dichloromethane R. Wash the combined dichloromethane extracts with two quantities 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 to dryness. Carry out the test as described under 1.7 Spectrophotometry in the infrared region. The infrared spectrum of the residue is concordant with the reference spectrum of trimethoprim RS.

**pH value (1.13).** pH of the oral suspension, 5.0 – 6.5.

**Related substances**

**Trimethoprim-related substances.** 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 chloroform R, 20 volumes of methanol R and 3 volumes of ammonia (~ 260 g/l) TS as the mobile phase. Prepare a solvent mixture as follows: mix 8 volumes of chloroform R and 2 volumes of methanol R. Apply separately to the plate 5 µl of each of the following three solutions. For solution (A), transfer a volume of oral suspension, containing about 40 mg of Trimethoprim to a separation funnel. Extract with three portions of 25 ml of the solvent mixture; collecting the extracts in a 125 ml conical flask. Evaporate to dryness the combined extracts with the aid of a current of air on a steam bath. Dissolve the residue in 2 ml of the solvent mixture, then centrifuge. For solution (B) use 20 mg of trimethoprim RS per ml of the solvent mixture. For solution (C), dilute an accurately measured volume of solution B with the solvent mixture to obtain a solution having a known concentration of 0.1 mg per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).
Trimethoprim produces a spot at about $R_F$ 0.7 and the trimethoprim degradation product produces a spot at about $R_F$ 0.3 to 0.5. Any spot obtained with solution A at about $R_F$ 0.3 to 0.5 is not greater in size and intensity than the spot obtained with solution C (0.5%).

**Sulfamethoxazole-related substances.** Carry out the test as described under 1.14.1. Thin-layer chromatography, using silica gel R5 as the coating substance. Prepare an ethanol-methanol solution by mixing 95 volumes of dehydrated ethanol R and 5 volumes of methanol R. As the mobile phase, use a mixture of 25 volumes of the ethanol-methanol solution, 25 volumes of heptane R, 25 volumes of dichloromethane R and 7 volumes of glacial acetic acid R. Apply separately to the plate 50 µl of each of the following three solutions. For solution (A), transfer a volume of the oral suspension containing 200 mg of Sulfamethoxazole to a 100 ml volumetric flask containing 10 ml of ammonia (~260 g/l) TS. Add 50 ml of methanol R, shake for 3 minutes, and dilute to volume with methanol R. Centrifuge a portion of the solution for 3 minutes. For solution (B), transfer 20 mg of sulfamethoxazole RS into a 10 ml volumetric flask, dissolve in 1 ml of ammonia (~260 g/l) TS and dilute to volume with methanol. For solution (C), transfer 10 mg of sulfanilamide RS into a 50 ml volumetric flask, dissolve in 5 ml of ammonia (~260 g/l) TS and dilute to volume with methanol R. Pipet 5 ml of this solution into a 100 ml volumetric flask, add 10 ml of ammonia (~260 g/l) TS and dilute to volume with methanol. For solution (D), transfer 10 mg of sulfanilic acid RS into a 50 ml volumetric flask, dissolve in 5 ml of ammonia (~260 g/l) and dilute to volume with methanol R. Pipet 3 ml of this solution into a 100 ml volumetric flask, add 10 ml of ammonia (~260 g/l) TS and dilute to volume with methanol R. For solution (E), transfer 3 mg of sulfamethoxazole $N_4$-glucoside RS into a 50 ml volumetric flask, dissolve in 5 ml of ammonia (~260 g/l) TS and dilute to volume with methanol R. For solution (F), transfer 10 mg of sulfanilamide RS, 6 mg of sulfanilic acid RS and 60 mg of sulfamethoxazole $N_4$-glucoside RS into a 100 ml volumetric flask, dissolve in 10 ml of ammonia (~260 g/l) TS and dilute to volume with methanol R. Pipet 1 ml of this solution into a 10 ml volumetric flask containing 20 mg of sulfamethoxazole RS, add 1 ml of ammonia (~260 g/l) TS and dilute to volume with methanol R to volume.

After removing the plate from the developing chamber, allow it to dry in air, spray with 4-dimethylaminobenzaldehyde TS7 and allow the plate to stand for 15 minutes.

Any spots corresponding to sulfanilamide, sulfanilic acid and sulfamethoxazole $N_4$-glucoside obtained with solution A are not greater in size and intensity than the spots obtained with solution C (0.5%), solution D (0.3%), and solution E (3.0%) respectively. The test is not valid unless the chromatogram obtained with solution F shows four clearly separated principal spots.

**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).\(^1\) 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 through a 0.45 μm membrane.

Prepare the following solutions. For solution (1) transfer an accurately weighed quantity of the oral suspension, containing about 80 mg of Sulfamethoxazole, to a 50-ml volumetric flask using about 30 ml of methanol R. Sonicate the mixture for about 10 minutes with occasional shaking. Allow to cool to room temperature, make up to volume with methanol R, mix and filter. Transfer 5.0 ml of clear filtrate into a 50 ml volumetric flask, make up to volume with the mobile phase and mix. For solution (2), use 0.32 mg of trimethoprim RS and 1.60 mg of sulfamethoxazole RS per ml of methanol R. Transfer 5.0 ml of this solution into a 50 ml volumetric flask, make up to volume 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 of solutions (1) and (2) and record the chromatogram for 1.5 times the retention time of sulfamethoxazole. The test is not valid unless the resolution factor between the peaks due to sulfamethoxazole and to trimethoprim is at least 5.0.

Determine the weight per ml (1.3.1) of the oral suspension and calculate the percentage content of Sulfamethoxazole (C\(_{10}\)H\(_{11}\)N\(_3\)O\(_3\)S) and Trimethoprim (C\(_{14}\)H\(_{18}\)N\(_4\)O\(_3\)) in the oral suspension from the declared content of Sulfamethoxazole (C\(_{10}\)H\(_{11}\)N\(_3\)O\(_3\)S) and Trimethoprim (C\(_{14}\)H\(_{18}\)N\(_4\)O\(_3\)) in sulfamethoxazole RS and trimethoprim RS.

B. To 8.0 g of the oral suspension, accurately weighed, add 30 ml of sodium hydroxide (~4 g/l) TS, shake and extract with four quantities of 50 ml of dichloromethane R, washing each extract with the same two quantities of 10 ml of sodium hydroxide (~4 g/l) TS. Reserve the combined dichloromethane extracts for the Assay method C. Dilute the combined aqueous solution and washings to 250 ml with water R, filter and dilute 5 ml of the filtrate to 200 ml with water R (solution A). Carry out the following procedure protected from light using 2 ml of solution A. Add 0.5 ml of hydrochloric acid (~146 g/l) TS and 1 ml of sodium nitrite (~1 g/l) TS and allow to stand for 2 minutes. Add 1 ml of ammonium sulfamate (~5 g/l) TS and allow to stand for 3 minutes. Add 1 ml of N-(1-napthyl)ethylenediamine hydrochloride (1 g/l) TS and allow to stand for 10 minutes. Dilute the resulting solution to 25 ml with water R and measure the absorbance at 538 nm, using in the reference cell a solution prepared in the same manner but using 2 ml of

---

\(^1\) Hypersil BDS C18 has been found suitable.
water R in place of solution A. Dissolve 0.25 g of sulfamethoxazole RS in 50 ml of sodium hydroxide (~4 g/l) TS and dilute to 250 ml with water R. Dilute 5 ml of the resulting solution to 200 ml with water R (solution B). Repeat the procedure described above, using 2 ml of solution B and starting at the sentence “Add 0.5 ml of hydrochloric acid (~146 g/l) TS and 1 ml of sodium nitrite (~ 1 g/l) TS ...”.

Calculate the content of Sulfamethoxazole (C_{10}H_{11}N_{3}O_{3}S) from the values of the absorbances obtained using the declared content of C_{10}H_{11}N_{3}O_{3}S in sulfamethoxazole RS. Determine the weight per ml (1.3.1) of the oral suspension, and calculate the content of Sulfamethoxazole (C_{10}H_{11}N_{3}O_{3}S), weight in volume.

C. Extract the dichloromethane solution reserved in the Assay for sulfamethoxazole with four quantities of 50 ml of acetic acid (~ 60 g/l) TS. Wash the combined extracts with 5 ml of dichloromethane R and dilute the aqueous extracts to 250 ml with acetic acid (~ 60 g/l) TS. To 10 ml of this solution add 10 ml of acetic acid (~ 60 g/l) TS and sufficient water R to produce 100 ml. Measure the absorbance of the resulting solution at the maximum at 271 nm.

Calculate the content of Trimethoprim (C_{14}H_{18}N_{4}O_{3}) using 204 as value for the specific absorbance ($\frac{A}{1%}$) at the maximum at 271 nm. Calculate the content of Trimethoprim (C_{14}H_{18}N_{4}O_{3}), weight in volume.

***

New reagents to be added in Ph.Int.

**Acetic acid (~10 g/l) TS**
Acetic acid (~300 g/l) TS, diluted with water to contain about 10 g of C_{2}H_{4}O per litre.

**Hydrochloric acid (~146 g/l) TS**
Hydrochloric acid (~250 g/l) TS, dilute with water to contain approximately 146 g of HCl in 1000 ml (approximately 4 mol/l).

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

**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.

***