SULFAMETHOXAZOLE AND TRIMETHOPRIM
INTRAVENOUS INFUSION
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/12.462

**Draft proposal for The International Pharmacopoeia: Sulfamethoxazole and Trimethoprim intravenous infusion**

<table>
<thead>
<tr>
<th>Description</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>First draft received from collaborating laboratory</td>
<td>February 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>
[Notes from the Secretariat:

This draft text is proposed for inclusion in Ph.Int. in the context of a collaboration between WHO and the Medicines and Healthcare products Regulatory Agency of the United Kingdom of Great Britain and Northern Ireland (MHRA) hosting The British Pharmacopoeia, on which this text is based.

- the use of "injection" or "infusion" to define this type of dosage form would need to be harmonized with either the EML where the term “injection” is used for this product, or with the existing monograph of a similar dosage form in the Ph.Int. (see Zidovudine intravenous infusion).]

Draft proposal for The International Pharmacopoeia

SULFAMETHOXAZOLI ET TRIMETHOPRIMI INFUSIO INTRAVENO
SULFAMETHOXAZOLE AND TRIMETHOPRIM INTRAVENOUS INFUSION

Category. Antibacterials.

Requirements

Complies with the monograph for “Parenteral preparation”.

Definition. Sulfamethoxazole and Trimethoprim intravenous infusion is a sterile solution of Sulfamethoxazole and Trimethoprim in glucose or sodium chloride intravenous infusions. It is prepared immediately before use by diluting Sulfamethoxazole and Trimethoprim sterile concentrate with a 5% glucose infusion or a 0.9% sodium chloride intravenous infusion.

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 containers, preferably of Type I glass.

[Note from the Secretariat: The BP monograph recommends the storage of the infusion in a “Type I glass container”; however, glass container categories are not described in Ph.Int. Moreover, as described in BP (Appendix XIX B refers – Glass containers for Pharmaceutical]
Type I glass containers are suitable for most preparations whether or not for parenteral use. As this type of glass container is in common use and does not have particular characteristics that would need to be specified in this monograph, it is proposed to omit this information and to mention the general term “glass container” instead.

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 ampoule
- 80 mg per ml Sulfamethoxazole, 16 mg per ml Trimethoprim in 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 ampoule
- 80 mg per ml Sulfamethoxazole, 16 mg per ml Trimethoprim in 10 ml ampoule.

Requirements

Comply with the monograph for “Parenteral preparations”.

Definition. Sulfamethoxazole and Trimethoprim sterile concentrate is a sterile solution of Sulfamethoxazole and Trimethoprim in water for injections, which, when diluted with a 5% glucose intravenous infusion or a 0.9% sodium chloride intravenous infusion, is suitable for intravenous infusion.

The solution is sterilized by a suitable method (see 5.8 Methods of sterilization).

Sulfamethoxazole and Trimethoprim sterile concentrate 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.

[Note from the Secretariat: It is intended to develop Ph.Int. monographs for Glucose intravenous infusion and Sodium chloride intravenous infusion.]

Identity tests

- Either tests A and D 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 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 the 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. The infrared absorption spectrum of the residue is concordant with the reference spectrum of sulfamethoxazole RS.

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 of 50 ml of dichloromethane R. Wash the combined 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 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 the principal peaks in the chromatogram obtained with solution (1) are similar to those in the chromatogram obtained with solution (2).

**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. Dilute the sterile concentrate with water BET to obtain a solution containing 1 mg of Trimethoprim and 5 mg of Sulfamethoxazole per ml (solution A). Solution A contains not more than 0.5 IU per ml.
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 concentrate, containing about 48 mg of Trimethoprim and 240 mg of Sulfamethoxazole, 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 extract 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 ml of a mixture of equal volumes of dichloromethane R and methanol R (solvent mixture). For solution (B) use 48 mg of sulfamethoxazole 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 of 240 µg 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 produces a spot at about Rf 0.5, and the trimethoprim degradation product produces a spot at about Rf 0.6 to 0.7. Any spot from solution A at about Rf 0.6 to 0.7 is not greater in size and intensity than the spot produced by solution C (0.5%). Disregard any spots due to concentrate excipients at about Rf 0.1.

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 30 volumes of ethanol-methanol solution, 30 volumes of heptane R, 30 volumes of dichloromethane R and 10 volumes of glacial acetic acid R. Prepare an ammonium hydroxide solution by diluting 1 ml of ammonia (~ 260 g/l) TS in the ethanol-methanol solution, and dilute to 100 ml with the same solution. Apply separately to the plate 10 µl of each of the following five solutions. For solution (A), transfer an accurately measured volume of concentrate, containing about 32 mg of Trimethoprim and 160 mg of Sulfamethoxazole, to an evaporating dish. Evaporate the sample to dryness using a steam bath. Reconstitute the residue with 16 ml of ammonium hydroxide solution. For solution (B) use 10 mg of sulfamethoxazole RS per ml of ammonium hydroxide solution. For solution (C) use 0.05 mg of sulfanilamide RS per ml of ammonium hydroxide solution. For solution (D) use 0.03 mg of sulfanilic acid RS per ml of ammonium hydroxide solution. For solution (E) dissolve 10 mg of sulfamethoxazole RS in 1 ml of a solution containing 0.05 mg of sulfanilamide RS and 0.03 mg of sulfanilic acid RS per ml of ammonium hydroxide solution. 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.

Any spots corresponding to sulfanilamide and sulfanilic acid in the chromatogram obtained with solution A are not greater in size or intensity than the spots obtained with solution C (0.5%) and solution D (0.3%) respectively. The test is not valid unless the chromatogram obtained with solution (E) shows three 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). 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 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), use 0.32 mg of trimethoprim RS and 1.60 mg of sulfamethoxazole RS per ml of 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. 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 (C_{10}H_{11}N_{3}O_{3}S) and Trimethoprim (C_{14}H_{18}N_{4}O_{3}) in the tablets.

B. To an accurately measured volume of the concentrate containing about 48 mg of Trimethoprim, add 30 ml of sodium hydroxide (~4 g/l) TS and extract with four

---

1 Hypersil BDS C18 has been found suitable.
quantities of 50 ml of dichloromethane R, washing each extract twice with a quantity of 10 ml of sodium hydroxide (~4g/l) TS. Combine the dichloromethane extracts and extract with four quantities of 50 ml of acetic acid (~60 g/l) TS. Wash the combined aqueous extracts with 5 ml of dichloromethane R and dilute to 250.0 ml with acetic acid (~60 g/l). To 10 ml of this solution, add 10 ml of acetic acid (~60 g/l), 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 (C\(_{14}\)H\(_{18}\)N\(_{4}\)O\(_{3}\)) using the absorptivity value of 20.4 (\(\text{A}^{1\%}_{1\text{cm}} = 204\)).

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 g/l) TS. Add 3 g of potassium bromide R, cool in ice and titrate slowly with sodium nitrite (0.1 mol/l) VS, stirring constantly and determining the end-point potentiometrically.

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

***

New reagents needed to be added to 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 (~3.65 g/l) TS**
Hydrochloric acid (~250 g/l) TS, dilute with water to contain 3.65g of HCl in 1000 ml.

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

**Hydrochloric acid (~36.5 g/l) TS**
Hydrochloric acid (~250 g/l) TS, dilute with water to contain 36.5g 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.