Revision of the Monograph on

PYRIMETHAMINE

(PYRIMETHAMINUM)

Draft revision for The International Pharmacopoeia

(June 2019)

DRAFT FOR COMMENTS

Please send any comments you may have to Dr Herbert Schmidt, Medicines Quality Assurance Programme, Technologies, Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland; fax: +41 22 791 4856 or email: schmidt@who.int by 31 August 2019.

In order to speed up the process for receiving draft monographs and for sending comments, please send your email address (to jonessi@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/17.696/Rev3:

**DRAFT REVISION FOR THE INTERNATIONAL PHARMACOPOEIA**

**PYRIMETHAMINE (PYRIMETHAMINUM)**

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
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<tr>
<td>First draft received from collaborating laboratory.</td>
<td>January 2017</td>
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<tr>
<td>Discussion at the informal Consultation on Screening Technology, Sampling and Specifications for Medicines.</td>
<td>May 2017</td>
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<tr>
<td>Revision of the first draft (Rev1).</td>
<td>September 2017</td>
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<tr>
<td>Submission to the 52(^{nd}) Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP).</td>
<td>October 2017</td>
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<tr>
<td>Draft revision (Rev2) sent out for public consultation.</td>
<td>November 2017 – January 2018</td>
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<tr>
<td>Discussion at the informal Consultation on Screening Technology, Sampling and Specifications for Medicines.</td>
<td>2-4 May 2018</td>
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<tr>
<td>Additional laboratory investigations and revision of the second draft in order to address comments received.</td>
<td>May 2018 – ongoing</td>
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<td>Submission to the 53(^{rd}) ECSPP.</td>
<td>October 2018</td>
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<tr>
<td>Discussion at the informal Consultation on Screening Technologies and Pharmacopoeial Specifications for Medicines.</td>
<td>2-3 May 2019</td>
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<tr>
<td>Draft revision (Rev3) sent out for public consultation.</td>
<td>July – August 2019</td>
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<td>Submission to the 54(^{th}) ECSPP.</td>
<td>October 2019</td>
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<td>Further follow-up action as required.</td>
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</table>

\[\textit{Note from the Secretariat: It is proposed to revise the monograph on Pyrimethamine in The International Pharmacopoeia.}\]
Pyrimethamine  
*(Pyrimethaminum)*

Molecular formula.  $\text{C}_{12}\text{H}_{13}\text{ClN}_4$

Relative molecular mass.  248.7

Graphic formula.

![Chemical structure of Pyrimethamine](image)

Chemical name.  5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine (*IUPAC*), 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (*CAS*); CAS Reg. No. 58-14-0.

Description.  An almost white, crystalline powder or colorless crystals.

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

Category.  Antimalarial.

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

Additional Information.  Pyrimethamine exhibits polymorphism.
Requirements

Definition. Pyrimethamine contains not less than 99.0% and not more than 101.0% of \( \text{C}_{12}\text{H}_{13}\text{ClN}_{4} \), 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 with pyrimethamine RS or with the reference spectrum of pyrimethamine.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and pyrimethamine RS in a small amount of dehydrated ethanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from pyrimethamine RS.

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under the “Related Substances” with the following modifications. Prepare the following solutions. For solution (1), use solution (1) as described under “Related Substances”. For solution (2), dissolve 12.5 mg pyrimethamine RS in about 20 mL solvent solution, sonicate for 10 minutes and dilute to 100.0 mL with mobile phase. Inject 30 μL of solutions (1) and (2). The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak corresponding to pyrimethamine in the chromatogram obtained with solution (2).

C. The absorption spectrum (1.6) of a 15 μg/mL solution in hydrochloric acid (0.005 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 272 nm and a minimum at about 261 nm.
Sulfates. Shake 1.0 g with 50 mL of distilled water for 2 minutes and filter. Proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.08 mg/g.

Sulfated Ash (2.3). Not more than 1.0 mg/g.

Loss on drying. Dry at 105°C for 4 hours; it loses not more than 5.0 mg/g.

Acidity or alkalinity. Boil 0.3 g with 15 mL of water, cool and filter. Add 0.25 mL of methyl red/ethanol TS to the filtrate; a yellow colour is observed. Not more than 0.1 mL of hydrochloric acid (0.05 mol/l) VS is required to change the colour of the solution to red.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (10 cm x 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3.5 µm). ¹

Prepare an ammonia solution by adding 10.0 mL of ammonia (~260 g/L) TS to 150 mL of water R, mix and dilute to 200.0 mL with water. Prepare an ammonium bicarbonate buffer pH 9.3 by dissolving 0.8 g of ammonium bicarbonate R in 1500 mL of water, adjust the pH to 9.3 by adding the ammonium solution (about 25 mL), mix and dilute to 2000.0 mL with water R.

As the mobile phase, use a mixture of 55 volumes of ammonium bicarbonate buffer pH 9.3 and 45 volumes of methanol R.

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 280 nm. Maintain the column temperature at 35°C.

¹ Agilent Zorbax Eclipse XDB-C18 has been found suitable.
Prepare as a solvent solution a mixture of 50 volumes of acetic acid (~ 10 g/L) TS and 50 volumes of methanol R.

Prepare the following solutions. For solution (1), weigh 25 mg of pyrimethamine into a 20 mL volumetric flask. Add approximately 15 mL of the solvent solution and sonicate for about 10 minutes. Dilute to volume with the solvent solution and mix. Dilute 5.0 mL of the filtrate to 50.0 mL with mobile phase. For solution (2), dilute 10.0 mL of solution (1) to 100.0 mL with mobile phase. For solution (3), prepare 20 mL of a 1.25 mg/mL solution of pyrimethamine in sulfuric acid (~570 g/L) TS in a 25 mL conical flask. Heat the solution on a hotplate until it boils. Continue to heat to reduce the volume to about half its initial volume. The final solution should be clear with a light tinge of yellow. Cool and dilute 1 volume of this solution to 10 volumes with mobile phase.

Inject 30 μL of solution (3).

Record the chromatogram for about 2.5 times the retention time of pyrimethamine (retention time about 12 minutes). The impurities are eluted, if present, at the following relative retention with reference to the pyrimethamine: impurity A about 0.35; impurity B about 0.45; impurity C about 0.64; impurity D about 0.15; impurity E about 0.42; impurity F about 0.52 and impurity G about 2.28. The test is not valid unless in the chromatogram obtained with solution (3) the resolution between impurities A and B is at least 3.0.

Inject alternately 30 μL of solutions (1) and (2).

Use the chromatogram obtained with solution (3) to identify the peaks due to the impurities A, B and C.

In the chromatogram obtained with solution (1):

- The area of any impurity peak is not greater than the area of the peak due to pyrimethamine in the chromatogram obtained with solution (2) (0.10%); and
The sum of the areas of all impurities is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%). Disregard any peak with an area less than 0.5 times the area of the peak due to pyrimethamine obtained with solution (2) (0.05%).

**Assay.** Dissolve about 0.20 g, accurately weighed, in 30 mL of anhydrous acetic acid R, heating gently. Cool and titrate with 0.1 M perchloric acid, determining the end-point potentiometrically. Each mL of 0.1 M perchloric acid is equivalent to 24.87 mg of C₁₂H₁₃ClN₄.

**Impurities.**

A. 4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2(1H)-one (degradation product).

B. 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4(1H,3H)-dione (degradation product).

C. 2-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(1H)-one (degradation product).
D. 2-(4-chlorophenyl)-3-oxopentanenitrile (synthesis-related impurity).

E. (4-chlorophenyl)acetonitrile (synthesis-related impurity).

F. 5-(4-chlorophenyl)-6-methylpyrimidine-2,4-diamine (synthesis-related impurity).

G. (2S)-4-chlorophenyl)(2-ethyl-1,3-dioxolan-2-yl)acetonitrile (synthesis-related impurity).

Reagents to be added to The International Pharmacopoeia:

- Ammonium bicarbonate R.
- Analytical reagent grade of commerce containing not less than 99% of NH₄HCO₃.