Consultation documents

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The International Pharmacopoeia

Pyrimethamine
(Pyrimethaminum)

This is a draft proposal of a revised monograph for The International Pharmacopoeia (Working document QAS/17.696/Rev.2, November 2017). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

(Note from the Secretariat: It is proposed to revise the monograph on Pyrimethamine in The International Pharmacopoeia.)

\[
\begin{array}{c}
\text{NH}_2 \\
\text{C}_2\text{H}_5 \\
\text{N} \\
\text{C}_6\text{H}_4\text{Cl} \\
\text{NH}_2 \\
\end{array}
\]

\[\text{C}_{12}\text{H}_{13}\text{ClN}_4\]

Relative molecular mass. 248.7

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

Description. A white, crystalline powder.

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 C₁₂H₁₃ClN₄, 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. 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 solution (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). The material contains embedded polar groups.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.

¹ Waters Xbridge Shield RP18 has been found suitable.
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.

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, accurately weighed, 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 solvent 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. Dilute 1.0 mL of this solution 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 times the retention time of pyrimethamine (retention time about 7 minutes). The impurities are eluted, if present, at the following relative retention with reference to the pyrimethamine: impurity A about 0.41; impurity B about 0.53 and impurity C about 0.69. The test is not valid unless in the chromatogram obtained with solution (3) the resolutions between impurities A and B is at least 3.0.

Inject alternately 30 μL of solution (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 peak corresponding to either impurity A, B or C is not greater than 2.5 times the area of the peak due to pyrimethamine in the chromatogram obtained with solution (2) (0.25%);
- the area of any other impurity peak is not greater than the area of the peak due to pyrimethamine in the chromatogram obtained with solution (2) (0.10%);
- the sum of the areas of all impurities is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). Disregard the peak due to pyrimethamine with an area less than 0.5 times the area of the principal peak obtained with solution (2) (0.05%).

Assay. Dissolve about 0.40 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 C_{12}H_{13}ClN_{4}. 

Pyrimethamine (Ph. Int.)
Impurities

A. 4-amino-5(4-chlorophenyl)-6-ethylpyrimidin-2(3H)-one (degradation product)

B. 2,4-dihydroxy-5-(4-chlorophenyl)-6-ethylpyrimidine) (degradation product)

C. 2-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-4(3H)-one (degradation product)

Reagents to be included:

Ammonium bicarbonate R
Analytical reagent grade of commerce containing not less than 99% of NH₄HCO₃.
Pyrimethamine tablets
(*Pyrimethamini compressi*)

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with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects).

[Note from the Secretariat: It is proposed to include the monograph on Pyrimethamine tablets in The International Pharmacopoeia.]

**Category.** Antimalarial.

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


**Requirements**

Comply with the monograph for Tablets.

**Definition.** Pyrimethamine tablets contain not less than 90.0% and not more than 110.0% of the labelled amount of pyrimethamine (C_{12}H_{13}ClN_{4}).

**Identity tests**

- Either test A or tests B and C may be applied.
  
  **A.** Shake a quantity of the powdered tablets, containing about 50 mg of pyrimethamine, with 50 mL of dehydrated ethanol R for 20 minutes, filter and evaporate the filtrate to dryness. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from pyrimethamine RS treated similarly.
  
  **B.** To a quantity of the powdered tablets, containing about 25 mg of pyrimethamine, add 50 mL of hot hydrochloric acid (~3.65 g/L) TS and heat on a water bath for 10 minutes, swirling occasionally. Mix with the aid of ultrasound for 30 minutes and cool to room temperature. Add sufficient hydrochloric acid (~3.65 g/L) TS to produce 100 mL. Filter a portion of this solution and discard the first few mL of the filtrate. Dilute 5 mL of the filtrate to 100 mL with hydrochloric acid (~3.65 g/L) TS. The absorption spectrum of the solution, when observed between 250 and 300 nm, exhibits a maximum at about 272 nm and a minimum at about 261 nm.
  
  **C.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the peak due to pyrimethamine in the chromatogram obtained with solution (2).
**Dissolution.** Carry out the test described under 5.5 Dissolution test for solid oral dosage forms using as the dissolution medium 900 mL of hydrochloric acid (~3.65 g/L) TS and rotating the paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10 mL of the medium through an in-line filter. Measure the absorbance (1.6) of a 1 cm layer of the filtered sample at the maximum at about 272 nm, using the dissolution buffer as the blank. Measure at the same time and under the same conditions the absorbance of a suitable solution of pyrimethamine RS in dissolution medium.

For each of the tablets tested, calculate the total amount of pyrimethamine \( \text{C}_{12}\text{H}_{13}\text{ClN}_4 \) in the medium from the results obtained. The amount in solution for each tablet is not less than 75% (Q) of the amount declared on the label.

[Note from the Secretariat: It is intended to determine the absorptivity value of pyrimethamine during the establishment of pyrimethamine RS and to use this value for the calculation of the test result.]

**Related substances.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay” with the following modifications:

For solution (2) dilute 10.0 mL of solution (1) to 100.0 mL with mobile phase. Dilute 1.0 mL of this solution 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 original 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 times the retention time of pyrimethamine (retention time about 7 minutes). The impurities are eluted at the following relative retention with reference to the pyrimethamine: impurity A about 0.41; impurity B about 0.53 and impurity C about 0.69. The test is not valid unless in the chromatogram obtained with solution (3) the resolutions between impurities A and B is at least 3.0.

Inject alternately 30 μL of solution (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 peak corresponding to either impurity A, B or C is not greater than 2.5 times the area of the peak due to pyrimethamine in the chromatogram obtained with solution (2) (0.25%).

**Assay.** 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). The material contains embedded polar groups.\(^1\)

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 the ammonium bicarbonate buffer pH

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\(^1\) Waters Xbridge Shield RP18 has been found suitable.
**Pyrimethamine tablets (Ph. Int.)**

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. 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 and powder 20 tablets. Transfer a quantity to the powdered tablets, containing about 125 mg of pyrimethamine, accurately weighed, into a 100 mL volumetric flask. Add approximately 75 mL of the solvent solution and sonicate for about 10 minutes. Dilute to volume with the solvent solution, mix and filter. Dilute 5.0 mL of the filtrate to 50.0 mL with mobile phase. For solution (2) dissolve 12.5 mg pyrimethamine RS in 20 mL, sonicate for 10 minutes and dilute to 100.0 mL with mobile phase.

Inject 30 µL of solution (1) and (2). Measure the areas of the peaks corresponding to pyrimethamine obtained in the chromatograms from solution (1) and (2) and calculate the percentage content of C_{12}H_{13}ClN_{4} in the tablets, using the declared content of C_{12}H_{13}ClN_{4} in pyrimethamine RS.

**Impurities**

The impurities limited by the requirements of this monograph include impurities A, B and C listed in the monograph on Pyrimethamine.

**Reagents to be included:**

**Ammonium bicarbonate R**

Analytical reagent grade of commerce containing not less than 99% of NH_{4}HCO_{3}.