## Pyrimethamine tablets (Pyrimethamini compressi)

## Category. Antimalarial.

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

Additional information. Strengths in the current WHO Model List of Essential Medicines (EML): 25 mg. Strength in the current WHO EML for children: 25 mg.

## Requirements

Comply with the monograph for <u>Tablets</u>.

**Definition.** Pyrimethamine tablets contain not less than 90.0% and not more than 110.0% of the labelled amount of Pyrimethamine ( $C_{12}H_{13}CIN_4$ ).

## Identity tests.

-Either test A or test B may be applied.

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 <u>1.7</u> <u>Spectrophotometry in the infrared region</u>. The infrared absorption spectrum is concordant with the spectrum obtained from pyrimethamine RS treated similarly.

Carry out test B.1 or, where a diode array detector is available, test B.2.

B.1 Carry out the test as described under <u>1.14.1 Chromatography</u>, <u>High-performance liquid chromatography</u> 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).

To a quantity of the powdered tablets, containing 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. Sonicate 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 (<u>1.6</u>) of the solution, when observed between 210 and 400 nm, shows a maximum at 272 nm.

B.2 Carry out the test as described under <u>1.14.1 Chromatography</u>, High-performance liquid chromatography using the conditions given under "Assay". Record the UV spectrum of the principle peak in the chromatograms with a diode array detector in the range of 210 nm to 400 nm. The retention time and the UV spectrum of the principal peak in the chromatogram obtained with solution (1) correspond to the retention time and UV spectrum of the peak corresponding to pyrimethamine in the chromatogram obtained with solution (2).

**Dissolution.** Carry out the test described under <u>5.5 Dissolution test for solid oral dosage forms</u>, 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 (<u>1.6</u>) of a 1-cm layer of the filtered sample at the maximum at about 272 nm, using the dissolution medium as the blank.

For each of the tablets tested, calculate the total amount of pyrimethamine (C12H13CIN4) in the medium using the absorptivity

 $A_{1 cm}^{1\%} = 316$ value of 31.6 for pyrimethamine ( $\frac{5.5 \text{ Dissolution test for solid}}{2}$ ). Evaluate the results as described under <u>5.5 Dissolution test for solid</u> oral dosage forms, Acceptance criteria. The amount of pyrimethamine released is not less than 75% (Q) of the amount declared on the label.

**Related substances**. Carry out the test as described under <u>1.14.1 Chromatography</u>, High-performance liquid chromatography using the conditions given under "Assay".

Use solution (1) as described under "Assay". Prepare the following additional solutions. 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 RS 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 of 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 2.5 times the retention time of pyrimethamine (retention time about 12 minutes). The impurities are eluted at the following relative retention with reference to 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

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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  $\mu$ L 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 peak corresponding to either impurity A, B or C is not greater than two times the area of the peak due to pyrimethamine in the chromatogram obtained with solution (2) (0.2%).

The sum of the areas of all impurity peaks is not greater than five times the area of the peak due to pyrimethamine in the chromatogram obtained with solution (2) (0.5%). Disregard any peak with an area less than the area of the peak due to pyrimethamine in the chromatogram in the chromatogram obtained with solution (2) (0.1%).

**Assay**. Carry out the test as described under <u>1.14.1 Chromatography</u>, <u>High-performance liquid chromatography</u> 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).

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 R. Prepare the ammonium bicarbonate buffer pH 9.3 by dissolving 0.8 g of ammonium bicarbonate R in 1500 mL of water R, adjust the pH to 9.3 by adding the ammonia solution (about 25 mL), mix and dilute to 200.0 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. For identity test B.2 use a diode array detector in the range of 210 nm to 400 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 of the powdered tablets, containing about 125.0 mg of pyrimethamine 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 of the solvent solution, sonicate for 10 minutes and dilute to 100.0 mL with mobile phase.

Inject 30  $\mu$ L of solutions (1) and (2).

Measure the areas of the peaks corresponding to pyrimethamine obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of  $C_{12}H_{13}CIN_4$  in the tablets, using the declared content of  $C_{12}H_{13}CIN_4$  in pyrimethamine RS.

**Impurities.** The related substances limited by the requirements of this monograph include those listed in the monograph on Pyrimethamine.