EAG/Panel/Working Party	Medicinal Chemicals 1
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Notes:

Revised monograph

Content limits revised from 92.5 – 107.5% to 95.0 – 105.0%.

Dissolution test added.

Related substances revised from TLC to LC, limits revised.

Assay revised from UV to LC.

Impurities section added.

Pyrimethamine Tablets

Pyrimethamine Preparations

Action and use
Dihydrofolate reductase inhibitor; antiprotozoal (malaria).

DEFINITION

Pyrimethamine Tablets contain Pyrimethamine.

The tablets comply with the requirements stated under Tablets and with the following requirements.

Content of pyrimethamine, $C_{12}H_{13}ClN_4$
95.0 to 105.0% of the stated amount

IDENTIFICATION

Shake a quantity of the powdered tablets containing 50 mg of Pyrimethamine with 50 mL of *ethanol (96%)* for 20 minutes, filter and evaporate the filtrate to dryness. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of pyrimethamine (*RS 309*).

TESTS

Dissolution
Comply with the requirements in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 50 revolutions per minute.

(b) Use 900 mL of 0.01M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 45 minutes withdraw a sample of the medium and dilute with the dissolution medium, if necessary, to produce a solution expected to contain 0.0028% w/v of Pyrimethamine and measure the *absorbance* at the maximum at 273 nm, Appendix II B, using dissolution medium in the reference cell.

(2) Measure the *absorbance* of a 0.0028% w/v solution of *pyrimethamine BPCRS* in the dissolution medium using dissolution medium in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of pyrimethamine, $C_{12}H_{13}ClN_4$, in the medium from the absorbances obtained and using the declared content of $C_{12}H_{13}ClN_4$ in *pyrimethamine BPCRS*.

LIMITS

The amount of pyrimethamine released is not less than 75% (Q) of the stated amount.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A: Equal volumes of 0.17M *acetic acid* and *methanol*.

(1) Swirl a quantity of the tablets containing 0.25 g of Pyrimethamine with 150 mL of solvent A until disintegrated. Mix with the aid of ultrasound and add sufficient solvent A to produce 200 mL. Filter (Whatman GMF is suitable) and dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) Dilute 1 volume of solution (1) to 100 volumes with mobile phase. Further dilute 1 volume to 10 volumes with mobile phase.

(3) 0.125% w/v of *pyrimethamine BPCRS* in 5.8M *sulfuric acid*. Boil 1 volume of the solution until reduced to approximately 0.5 volumes. The resulting solution should be clear and slightly yellow. Allow to cool and dilute 1 volume to 10 volumes with mobile phase (generation of impurities 1, 2 & 3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *ethylene-bridged octadecylsilyl silica gel for chromatography (hybrid material)* (3.5 µm) (Waters XBridge Shield RP18 is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.5 mL per minute.

(d) Use a column temperature of 35°.

(e) Use a detection wavelength of 280 nm.

(f) Inject 30 µL of each solution.

(g) Allow the chromatography to proceed for twice the retention time of pyrimethamine.

MOBILE PHASE

45 volumes of *methanol* and 55 volumes of 0.005M *ammonium hydrogen carbonate*, adjusted to pH 9.3 with 0.74M *ammonium hydroxide*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to pyrimethamine (retention time about 7 minutes) are; impurity 1, about 0.4; impurity 2, about 0.5 and impurity 3, about 0.7.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 1 and impurity 2 is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the area of any *secondary peaks* is not greater 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

ASSAY

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solvent A: Equal volumes of 0.17M *acetic acid* and *methanol*.

(1) Mix with the aid of ultrasound, a quantity of the powdered tablets containing 0.25 g of Pyrimethamine in 150 mL of solvent A and dilute with solvent A to produce 200 mL. Filter (Whatman GMF is suitable) and dilute 1 volume of the filtrate to 10 volumes with the mobile phase.

(2) 0.0125% w/v of *pyrimethamine BPCRS* in mobile phase.

(3) 0.125% w/v of *pyrimethamine BPCRS* in 5.8M *sulfuric acid*. Boil 1 volume of the solution until reduced to approximately 0.5 volumes. The resulting solution should be clear and slightly yellow. Allow to cool and dilute 1 volume to 10 volumes with mobile phase (generation of impurities 1, 2 & 3).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity 1 and impurity 2 is at least 3.0.

DETERMINATION OF CONTENT

Calculate the content of pyrimethamine, $C_{12}H_{13}ClN_4$, in the tablets using the declared content of $C_{12}H_{13}ClN_4$ in *pyrimethamine BPCRS*.

IMPURITIES

The impurities limited by the requirements of this monograph include:

1. 2-amino-5-(4-chlorophenyl)-6-ethyl-1,4-dihydropyrimidin-4-one;
2. 5-(4-chlorophenyl)-6-ethyl-1,2,3,4-tetrahydropyrimidin-2,4-dione;
3. 4-amino-5-(4-chlorophenyl)-6-ethyl-1,2-dihydropyrimidin-2-one.