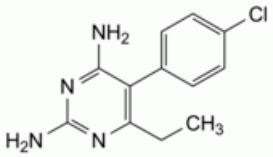
Edition: BP 2025 (Ph. Eur. 11.6 update)

Pyrimethamine

General Notices

(Ph. Eur. monograph 0288)



$C_{12}H_{13}ClN_4$ 248.7 *58-14-0*

Action and use

Dihydrofolate reductase inhibitor; antiprotozoal (malaria).

Preparation

Pyrimethamine Tablets

Ph Eur

DEFINITION

5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (2.2.14): 239 °C to 243 °C.
B. Infrared absorption spectrophotometry (2.2.24).

Comparison pyrimethamine CRS.

C. Thin-layer chromatography (2.2.27).

Solvent mixture methanol R, methylene chloride R (10:90 *V/V*).

Test solution Dissolve 0.1 g of the substance to be examined in the solvent mixture and dilute to 100 mL with the solvent mixture.

Reference solution Dissolve 0.1 g of pyrimethamine CRS in the solvent mixture and dilute to 100 mL with the solvent mixture.

Plate TLC silica gel F_{254} plate R.

Mobile phase methylene chloride R, propanol R, glacial acetic acid R, toluene R (4:8:12:76 *V/V/V/V*).

Application 20 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Solution S

Shake 1.0 g with 50 mL of carbon dioxide-free water R for 2 min and filter.

Appearance of solution

Prepare the solution immediately before use Dissolve 0.25 g in a mixture of 1 volume of methanol R and 3 volumes of methylene chloride R and dilute to 10 mL with the same mixture of solvents. The solution is clear (2.2.1) and not more intensely coloured than reference solution BY_6 (2.2.2, Method II).

Acidity or alkalinity

To 10 mL of solution S add 0.05 mL of phenolphthalein solution R1. The solution is colourless. Not more than 0.2 mL of 0.01 M sodium hydroxide is required to change the colour of the indicator to pink. Add 0.4 mL of 0.01 M hydrochloric acid and 0.05 mL of methyl red solution R. The solution is red or orange.

Related substances

Liquid chromatography (2.2.29).

Solvent mixture Mobile phase A, mobile phase B (50:50 *V/V*).

Test solution Dissolve 10.0 mg of the substance to be examined in 4 mL of the solvent mixture using sonication and dilute to 10.0 mL with the solvent mixture.

Reference solution (a) Dissolve 10 mg of the substance to be examined and 10 mg of pyrimethamine impurity B CRS in 50 mL of the solvent mixture using sonication and dilute to 100 mL with the solvent mixture.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dissolve 2.72 g of *potassium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust to pH 8.0 with *ammonia R* and dilute to 1000 mL with *water for chromatography R*;

— *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 6	65	35
6 - 20	65 → 40	35 → 60
20 - 35	40 → 55	60 → 45

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B.

Relative retention With reference to pyrimethamine (retention time = about 15 min): impurity B = about 0.8.

System suitability Reference solution (a):

— *resolution*: minimum 5.0 between the peaks due to impurity B and pyrimethamine.

Calculation of percentage contents:

— for each impurity, use the concentration of pyrimethamine in reference solution (b).

Limits:

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.3 per cent;

— *reporting threshold*: 0.05 per cent.

Sulfates (*2.4.13*)

Maximum 80 ppm, determined on solution S. Prepare the standard using a mixture of 2.5 mL of *sulfate standard solution (10 ppm SO_4) R* and 12.5 mL of *distilled water R*.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 4 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in 25 mL of *anhydrous acetic acid R* with gentle heating and allow to cool. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

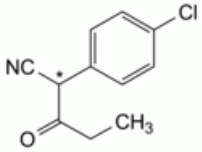
1 mL of *0.1 M perchloric acid* is equivalent to 24.87 mg of $C_{12}H_{13}ClN_4$.

STORAGE

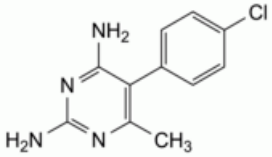
Protected from light.

IMPURITIES

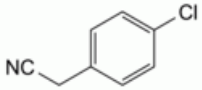
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, B, C, D.*



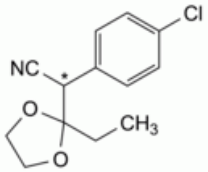
A. (2Ξ)-2-(4-chlorophenyl)-3-oxopentanenitrile,



B. 5-(4-chlorophenyl)-6-methylpyrimidine-2,4-diamine,



C. (4-chlorophenyl)acetonitrile,



D. (Ξ)-(4-chlorophenyl)(2-ethyl-1,3-dioxolan-2-yl)acetonitrile.

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