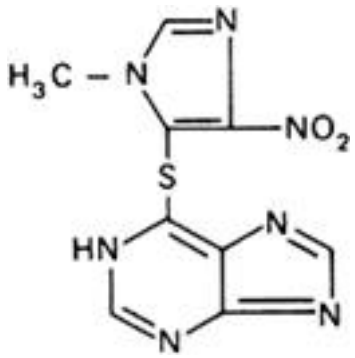
Azathioprine (Azathioprinum)

Molecular formula. $C_9H_7N_7O_2S$

Relative molecular mass. 277.3

Graphic formula.



Chemical name. 6-[(1-Methyl-4-nitroimidazol-5-yl)thio]purine; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine; CAS Reg. No. 446-86-6.

Description. A pale yellow powder; odourless.

Solubility. Practically insoluble in water; very slightly soluble in ethanol (~750 g/l) TS; sparingly soluble in dilute mineral acids; soluble in dilute solutions of alkali hydroxides.

Category. Immunosuppressive drug.

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

Additional information. Azathioprine decomposes in strong solutions of alkali hydroxides. CAUTION: Azathioprine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Azathioprine contains not less than 98.0% and not more than 101.5% of $C_9H_7N_7O_2S$, 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 from azathioprine RS or with the *reference spectrum* of azathioprine.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Heat 20 mg with 100 mL of water and filter. To 5 mL of the nitrate add 1 mL of hydrochloric acid (~420 g/l) TS, 10 mg of zinc R powder, and allow to stand for 5 minutes; the solution becomes yellow. Filter, cool in ice, add 0.1 mL of sodium nitrite (100 g/l) TS and 0.1 g of sulfamic acid R, and shake until the bubbles disappear. Add 1 mL of 2-naphthol TS1; a pale pink precipitate is produced.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 5 hours; it loses not more than 10 mg/g.

Acidity or alkalinity. Shake 0.5 g with 25 mL of water for 15 minutes, and filter; to 20 mL of the filtrate add 0.15 mL of methyl red/ethanol TS; not more than 0.10 mL of hydrochloric acid (0.02 mol/l) VS or 0.10 mL of sodium hydroxide (0.02 mol/l) VS is required to obtain the midpoint of the indicator (orange).

Related substances. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using cellulose R3 (a precoated plate from a commercial source is suitable) and 1-butanol R saturated with ammonia (~100 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in ammonia (~100 g/l) TS containing (A) 10 mg of the test substance per mL, (B) 0.15 mg of the test substance per mL, and (C) 0.15 mg of azathioprine RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any

spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 50 mL of dimethylformamide R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method B. Each mL of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 27.73 mg of $C_9H_7N_7O_2S$.