**Acidity or alkalinity.** Dissolve 0.4 g in 10 ml of water; to a 2-ml portion add 0.1 ml of bromocresol green/ethanol TS; the colour of the solution is blue. To another 2-ml portion of the test solution add 0.1 ml of phenol red/ethanol TS; the colour of the solution is yellow.

**Foreign salts.** Dissolve 1.2 g in 30 ml of water, add 7.5 ml of hydrochloric acid (~70 g/l) TS, shake vigorously, heat on a water-bath for 5 minutes and filter. Evaporate 20 ml of the filtrate to dryness on a water-bath and dry at 105 °C; the residue weighs not more than 2.0 mg.

**Bismuth, copper, and lead.** Dissolve 1.0 g in 5 ml of water, add drop by drop ammonia (~100 g/l) TS until the precipitate first formed just dissolves; the solution is clear and colourless.

**Assay.** Dissolve about 0.3 g, accurately weighed, in 50 ml of water, add 2 ml of nitric acid (~130 g/l) TS and 4 ml of ferric ammonium sulfate (45 g/l) TS. Titrate with ammonium thiocyanate (0.1 mol/l) VS until a reddish yellow colour is produced. Each ml of ammonium thiocyanate (0.1 mol/l) VS is equivalent to 16.99 mg of $AgNO_3$.

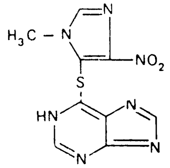
# AZATHIOPRINUM

## Azathioprine

**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 and chloroform R; sparingly soluble in dilute mineral acids; soluble in dilute solutions of alkali hydroxides.

of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 359 nm. Calculate the amount of $C_6H_6N_4O_3S$ in the substance being tested by comparison with niridazole RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of a 10 µg/ml solution of niridazole RS should be 0.70 ± 0.03.

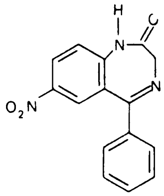
# NITRAZEPAMUM

## Nitrazepam

**Molecular formula.** $C_{15}H_{11}N_3O_3$

**Relative molecular mass.** 281.3

**Graphic formula.**



**Chemical name.** 1,3-Dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one; CAS Reg. No. 146-22-5.

**Description.** A yellow, crystalline powder; odourless or almost odourless.

**Solubility.** Practically insoluble in water; sparingly soluble in chloroform R; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

**Category.** Sedative; hypnotic.

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

REQUIREMENTS

**General requirement.** Nitrazepam contains not less than 98.5% and not more than 101.0% of $C_{15}H_{11}N_3O_3$, calculated with reference to the dried substance.

**Related substances.** Carry out the test as described under "Thin-layer chromatography" (vol. 1, p. 83), using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and 20 volumes of chloroform R, 10 volumes of hexane R, 2 volumes of 2-propanol R, and 0.5 volume of isopropylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of equal volumes of chloroform R and methanol R containing (A) 25 mg of the test substance per ml and (B) 0.25 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air for 10 minutes until the solvents have evaporated. Examine the chromatogram in ultraviolet light first at 254 nm then at 365 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 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, previously neutralized to oracet blue R/acetic acid TS. Titrate with perchloric acid (0.1 mol/l) VS as described under "Non-aqueous titration", Method A (vol. 1, p. 131). Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.93 mg of $C_{14}H_{21}N_3O_3$.

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# OXYTETRACYCLINI DIHYDRAS

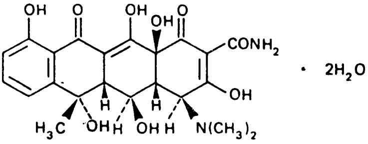
## Oxytetracycline dihydrate

### Oxytetracycline dihydrate (non-injectable)
### Oxytetracycline dihydrate, sterile

**Molecular formula.** $C_{2}H_{24}N_2O_9,2H_2O$

**Relative molecular mass.** 496.5

**Graphic formula.**



**Chemical name.** (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide dihydrate; [4*S*-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2naphthacenecarboxamide dihydrate; CAS Reg. No. 6153-64-6 (dihydrate).

**Identity tests**

A. Dissolve 0.05 g in 100 ml of methanol R. To 1 ml of this solution add 0.5 ml of hydrochloric acid (0.1 mol/l) VS and dilute to 100 ml with methanol R. Protect the solution from light and immediately measure the absorbance of a 1-cm layer at the maximum wavelength of about 249 nm; about 0.88.

B. Dissolve 0.1 g in 10 ml of water and add 0.05 ml of ferric chloride (25 g/l) TS; a violet-blue colour is produced.

C. Boil 0.1 g with 1 ml of hydrochloric acid (~70 g/l) TS for 3 minutes, add 10 ml of water and cool; no precipitate is formed. Add 0.05 ml of potassium dichromate (0.0167 mol/l) VS; a violet colour, which does not turn to red (distinction from phenacetin) is slowly produced.

**Melting range.** 168 – 172 °C.

**Heavy metals.** Use 1.0 g and a mixture of 85 volumes of acetone R and 15 volumes of water for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 2 (vol. 1, p. 118); determine the heavy metals content according to Method A (vol. 1, p. 119); not more than 10 µg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

**4-Aminophenol.** Dissolve 0.5 g in a mixture of equal volumes of methanol R and water and dilute to 10 ml with this solvent mixture. Add 0.2 ml of alkaline sodium nitroprusside TS, mix, and allow to stand for 30 minutes. Prepare similarly a reference solution containing 0.5 g of 4-aminophenol-free paracetamol R and 0.5 ml of a solution containing 0.050 mg/ml of 4-aminophenol R in the same solvent mixture. The colour of the test solution is not more intense than that of the reference solution (0.05 mg/g).

**Related substances.** Carry out the test as described under "Thin-layer chromatography", using silica gel R4 as the coating substance and a mixture of 65 volumes of chloroform R, 25 volumes of acetone R, and 10 volumes of toluene R as the mobile phase. Allow the solvent front to ascend 14 cm above the line of application, using an unlined chromatographic chamber. Prepare the following 4 test solutions: For solution (A) transfer 1.0 g of finely powdered substance to be examined to a glass-stoppered tube, add 5 ml of ether R, and shake mechanically for 30 minutes. Centrifuge the tube until a clear supernatant liquid is obtained and separate this from the solid. For solution (B) dilute 1 ml of solution A to 10 ml with ethanol (~750 g/l) TS. For solution (C) dissolve 25 mg of 4-chloroacetanilide R in 50 ml of ethanol (~750 g/l) TS. For solution (D) dissolve 0.25 g of 4-chloroacetanilide R and 0.1 g of the substance to be examined in sufficient ethanol (~750 g/l) TS to produce 100 ml. Apply separately to the plate 200 µl of solution A