Oxytetracycline hydrochloride (Oxytetracyclini hydrochloridum)

Oxytetracycline hydrochloride (non-injectable)

Oxytetracycline hydrochloride, sterile

Molecular formula. C\textsubscript{22}H\textsubscript{24}N\textsubscript{2}O\textsubscript{9},HCl

Relative molecular mass. 496.9

Graphic formula.

Chemical name. (4S,4aR,5S,5aR,6S,12aS)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; \([4S-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride;\) CAS Reg. No. 2058-46-0.

Description. A yellow, crystalline powder; odourless.

Solubility. Soluble in 2 parts of water and in 45 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antibacterial drug.

Storage. Oxytetracycline hydrochloride should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Oxytetracycline hydrochloride indicates that the substance complies with the additional requirements for sterile Oxytetracycline hydrochloride and may be used for parenteral administration or for other sterile applications.

Additional information. Oxytetracycline hydrochloride is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Dissolved in water it becomes turbid on standing owing to the separation of the base caused through partial hydrolysis of the hydrochloride. It deteriorates in solutions of pH below 2, and is rapidly destroyed by alkali hydroxide solutions.

Requirements

Definition. Oxytetracycline hydrochloride contains not less than 870 International Units of Oxytetracycline per mg, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a kieselguhr coating prepared as follows: To 25 g of kieselguhr R1 add 50 mL of a mixture of 2.5 mL of glycerol R and 47.5 mL of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS. Coat the plates with this mixture, and allow them to dry at room temperature for about 70-90 minutes, or until sufficiently dry to give a satisfactory separation. As the mobile phase, take 200 mL of a mixture of 2 volumes of ethyl acetate R, 2 volumes of chloroform R, and 1 volume of acetone R, shake with 25 mL of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS, allow to settle, and use the lower layer. Apply separately to the plate 1 μl of each of 3 solutions in methanol R containing (A) 0.50 mg of the test substance per mL, (B) 0.50 mg of Oxytetracycline hydrochloride RS per mL, and (C) a mixture of 0.50 mg of chlortetracycline hydrochloride RS per mL, 0.50 mg of Oxytetracycline hydrochloride RS per mL, and 0.50 mg of tetracycline hydrochloride RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows 3 clearly separated spots.

B. To about 1 mg add 2 mL of sulfuric acid (~1760 g/l) TS; a deep red colour is produced, which changes to yellow on the addition of 0.1 mL of water.

C. A 0.05 g/mL solution yields reaction B described under 2.1 General identification tests as characteristic of...
chlorides.

**Specific optical rotation.** Dissolve 0.25 g in sufficient hydrochloric acid (0.1 mol/l) VS to produce 25 mL and allow to stand for 60 minutes. Measure the rotation and calculate with reference to the anhydrous substance; \([\alpha]_{b}^{20\circ}\) = -188° to -200°.

**Sulfated ash.** Not more than 5.0 mg/g.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.25 g of the substance; the water content is not more than 20 mg/g.

**pH value.** pH of a 10 mg/mL solution, 2.0-3.0.

**Absorption in the ultraviolet region.** The absorption spectrum of a 20 μg/mL solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 400 nm, exhibits 2 maxima at about 268 nm and 353 nm. The absorbance of a 1-cm layer at 353 nm is not less than 0.54 and not more than 0.58.

**Light-absorbing impurities.** Prepare a 2.0 mg/mL solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R and measure within 1 hour of preparation the absorbance of a 1-cm layer at 430 nm; the absorbance does not exceed 0.50. Prepare a 10 mg/mL solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R and measure within 1 hour of preparation the absorbance of a 1-cm layer at 490 nm; the absorbance does not exceed 0.20.

**Assay.** Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (a) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 6.5-6.6, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of oxytetracycline (usually between 2 and 20 IU per mL), and an incubation temperature of 37-39°C, or (b) *Bacillus cereus* (ATCC 11778) as the test organism, culture medium Cm1 with a final pH of 5.9-6.0, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of oxytetracycline (usually between 0.5 and 2 IU), and an incubation temperature of 30 - 33°C. The precision of the assay is such that the fiducial limits of error of the estimated potency \((P = 0.95)\) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency \((P = 0.95)\) is not less than 870 IU of oxytetracycline per mg, calculated with reference to the anhydrous substance.

**Additional Requirements for Oxytetracycline Hydrochloride for sterile use**

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.4 IU of endotoxin RS per mg of oxytetracycline.

**Sterility.** Complies with 3.2 Test for sterility.