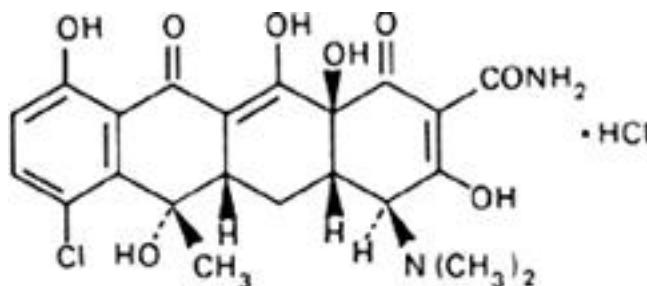


Chlortetracycline hydrochloride (Chlortetracyclini hydrochloridum)**Chlortetracycline hydrochloride (non-injectable)****Chlortetracycline hydrochloride, sterile****Molecular formula.** $C_{22}H_{23}ClN_2O_8 \cdot HCl$ **Relative molecular mass.** 515.4**Graphic formula.**

Chemical name. (4S,4aS,5aS,6S,12aS)-7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; [4S-(4a,4aa,5aa,6β,12aa)]-7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; CAS Reg. No. 64-72-2.

Description. Yellow crystals or a yellow, crystalline powder; odourless.

Solubility. Soluble in about 100 parts of water and in about 250 parts of ethanol (~760 g/l) TS; practically insoluble in acetone R and ether R.

Category. Antiinfective drug.

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

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

Additional information. Chlortetracycline hydrochloride has a bitter taste. Even in the absence of light, Chlortetracycline hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Chlortetracycline hydrochloride contains not less than 900 International Units of Chlortetracycline per mg, calculated with reference to the dried 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 chlortetracycline 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. Dissolve 10 mg in 10 mL of phosphate buffer, pH 7.6, TS, heat at 100°C for 1 minute and examine the solution in ultraviolet light (365 nm); a strong blue fluorescence is observed.

C. To about 1 mg add 2 mL of sulfuric acid (~1760 g/l) TS; a blue to bluish green colour is produced, which

changes to brown on the addition of about 1 mL of water.

D. A 0.05 g/mL solution yields reaction B described under [2.1 General identification tests](#) as characteristic of chlorides.

Specific optical rotation. Dissolve 0.125 g in sufficient water to produce 25.0 mL, and allow to stand in the dark for 30 minutes.

Measure the rotation at 25 °C and calculate with reference to the dried substance; $[\alpha]_D^{25^\circ} = -235^\circ \text{ to } -250^\circ$.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under [2.2.3 Limit test for heavy metals](#), Procedure 3; determine the heavy metals content according to Method A; not more than 50 µg/g.

Sulfated ash. Not more than 5.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 3 hours; it loses not more than 20 mg/g.

pH value. pH of a 10 mg/mL solution, 2.3-3.3.

Absorption in the ultraviolet region. Dissolve 10 mg in sufficient sulfuric acid (0.5 mol/l) VS to produce 100 mL. Dilute 10 mL of this solution to 100 mL with sulfuric acid (0.5 mol/l) VS. Place 10 mL of the resulting solution in a test-tube 25 mm in diameter and 200 mm long and immerse in a water-bath for 8 minutes. Cool, replace the water lost by evaporation, and measure the absorbance of a 1-cm layer at 274 nm; not less than 0.70 and not more than 0.76.

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 6.0-6.2, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of chlortetracycline (usually between 2 and 20 IU per mL), and an incubation temperature of 35-39°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 chlortetracycline (usually between 0.05 and 0.2 IU), and an incubation temperature of 29-33 °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 900 IU of chlortetracycline per mg, calculated with reference to the dried substance.

Additional Requirements for Chlortetracycline Hydrochloride for sterile use

Storage. Sterile Chlortetracycline hydrochloride should be kept in a hermetically closed container, protected from light.

Bacterial endotoxins. Carry out the test as described under [3.4 Test for bacterial endotoxins](#); contains not more than 1.0 IU of endotoxin RS per mg.

Sterility. Complies with [3.2 Test for sterility](#).