DOXYCYCLINE HYCLATE

Proposal for revision of published monograph in
The International Pharmacopoeia

(October 2006)

DRAFT FOR DISCUSSION

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## SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/06.185

*Revision of International Pharmacopoeia monograph on Doxycycline hyclate*

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<td>Discussion during the Consultation on specifications for medicines and quality control laboratory issues in connection with the monograph for doxycycline hyclate capsules.</td>
<td>25-27 July 2006</td>
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<td>Exchange of correspondence with WHO Collaborating Centres and experts to prepare a draft text</td>
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<td>Discussion of proposed revision at WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
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DOXYCYCLINE HYCLATE:
Draft proposal for revision of published monograph in
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Proposal for revision of published monograph

Doxycyclini hyclas - Doxycycline hyclate

Molecular formula. \((\text{C}_{22}\text{H}_{24}\text{N}_{2}\text{O}_{8}\cdot\text{HCl})_{2}\cdot\text{C}_{2}\text{H}_{6}\text{O}\cdot\text{H}_{2}\text{O}\)

Relative molecular mass. 1026

Graphic formula.

Chemical name. \((4S,4\alpha R,5S,5\alpha R,6R,12aS)-4-(\text{Dimethylamino})-1,4,4\alpha,5,5\alpha,6,11,12a\text{-octahydro}-3,5,10,12,12a\text{-pentahydroxy}-6\text{-methyl}-1,11\text{-dioxo}-2\text{-naphthacenecarboxamide monohydrochloride, compound with ethyl alcohol (2:1), mono-hydrate; [4S-}(4\alpha,4\alpha,5\alpha,5\alpha,6\alpha,12a\alpha)]-4-(\text{dimethylamino})-1,4,4\alpha,5,5\alpha,6,11,12a\text{-octahydro}-3,5,10,12,12a\text{-pentahydroxy}-6\text{-methyl}-1,11\text{-dioxo}-2\text{-naphthacenecarboxamide monohydrochloride, compound with ethanol (2:1), monohydrate; CAS Reg. No. 24390-14-5.}

Description. A yellow, crystalline powder.
**Solubility.** Soluble in 3 parts of water and in 4 parts of methanol R; practically insoluble in ether R.

**Category.** Antibacterial drug.

**Storage.** Doxycycline hyclate should be kept in a tightly closed container, protected from light.

**Additional information.** Even in the absence of light, Doxycycline hyclate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

**Requirements**

**Definition.** Doxycycline hyclate contains not less than 880 International Units of doxycycline per mg 95% and not more than 102% of \( \text{C}_{22}\text{H}_{24}\text{N}_{2}\text{O}_{8}\cdot\text{HCl} \), calculated with reference to the anhydrous and ethanol-free substance.

**Identity tests**

A. Dissolve 5 mg in 2 ml of sulfuric acid (~1760 g/l) TS; an intense yellow colour is produced.

B. Dissolve 5 mg in 2.0 ml of water and add 0.05 ml of ferric chloride (~25 g/l) TS; a dark red-brown colour is produced.

C. Dissolve 5 mg in 2.0 ml of water and add 0.25 ml of alkaline potassio-mercuric iodide TS; a fine crystalline, light yellow precipitate is formed.

D. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

**Sulfated ash.** Not more than 4.0 mg/g.
**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method. Method A, using about 1.2 g of the substance; the water content is not less than 14 mg/g and not more than 28 mg/g.

**Ethanol.** Carry out the test as described under 1.14.5 Gas chromatography, using 3 solutions in water containing (1) a mixture of 0.50 µl of dehydrated ethanol R per ml, (2) 10 mg of the test substance per ml, and (3) a mixture of 10 mg of the test substance per ml with 0.50 µl of the internal standard.

For the procedure use a column 1.5 m long and 4 mm in internal diameter packed with porous polymer beads (particle size 80-100 µm from a commercial source is suitable). Maintain the column at 135°C, use nitrogen R as the carrier gas and a flame ionization detector.

Calculate the content of ethanol in mg/g, assuming the weight per ml at 20°C to be 0.790 g; not less than 43 mg/g and not more than 60 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 10 µg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R exhibits a maximum at about 349 nm. The absorbance of a 1-cm layer at this maximum is not less than 0.28 and not more than 0.31 for the anhydrous and ethanol-free substance.

**Light-absorbing impurities.** 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 the absorbance of a 1-cm layer at 490 nm; the absorbance does not exceed 0.12 for the anhydrous and ethanol-free substance.

**Related substances.** Carry out the test as described under 1.14.4 High performance liquid chromatography, using the chromatographic conditions and preparing the solutions as described under Assay.

Inject 20µl of solution (5). The test is not valid unless the resolution between the first peak (metacycline) and the second peak (6-epidoxycycline) is greater than 1.25, and the
resolution between the second peak and the third peak (doxycycline) is greater than 2.0. If necessary, adjust the tert-butanol R content in the mobile phase. The test is not valid unless the symmetry factor for the third peak is less than 1.25. If necessary adjust the integrator parameters.

In the chromatogram obtained with solution (1) the area of any peak corresponding to metacycline or to 6-epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with solution (5) (2% with reference to doxycycline hyclate), the area of any peak appearing between the solvent peak and the peak corresponding to metacycline and the area of any peak appearing on the tail of the main peak is not greater than 0.25 times the area of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with solution (5) (0.5% with reference to doxycycline hyclate).

**Assay.** Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Bacillus cereus* (NCTC 10220 or ATCC 11778) as the test organism, culture medium Cm10 with a final pH of 6.6, potassium dihydrogen phosphate (13.6 g/l) TS as a buffer, an appropriate concentration of doxycycline (usually between 0.2 and 2.0 IU per ml), and an incubation temperature of 35–39 °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 880 IU of doxycycline per mg, calculated with reference to the anhydrous and ethanol-free substance.

Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25cm × 4.6mm) packed with particles of styrene-divinylbenzene copolymer (8 - 10 µm). As the mobile phase, use a solution prepared as follows: transfer 60.0g of tert-butanol R with the aid of 200ml of water to a 1000-ml volumetric flask. Add 400ml of buffer borate, pH 8.0, TS, 50ml of a solution of 10mg of tetrabutylammonium hydrogen sulfate R per ml adjusted to pH 8.0 with sodium hydroxide (~80g/l) TS, and 20ml of sodium edetate (20g/l) TS adjusted to pH 8.0 with sodium hydroxide (~80g/l) TS. Dilute to 1000ml with water.
Prepare the following solutions in hydrochloric acid (0.01mol/l) VS. For solution (1) use an accurately weighed amount sufficient to produce a solution containing the equivalent of 0.70mg of doxycycline per ml. Solution (2) contains 0.80mg of doxycycline hyclate RS per ml, solution (3) 0.80mg of 6-epidoxycycline hydrochloride RS per ml, solution (4) 0.80mg of metacycline hydrochloride RS per ml. For solution (5) mix 4.0ml of solution (2) with 1.5ml of solution (3) and 1.0ml of solution (4), and dilute to 25ml with hydrochloric acid (0.01mol/l) VS and for solution (6) mix 2.0ml of solution (3) and 2.0ml of solution (4) and dilute to 100ml with hydrochloric acid (0.01mol/l) VS.

Operate with a flow rate of about 0.9ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254nm.

Inject 20 µl of solution (5). The test is not valid unless the resolution between the first peak (metacycline) and the second peak (6-epidoxycycline) is greater than 1.25, and the resolution between the second peak and the third peak (doxycycline) is greater than 2.0. If necessary, adjust the tert-butanol R content in the mobile phase. The test is not valid unless the symmetry factor for the third peak is less than 1.25. If necessary adjust the integrator parameters.

Inject alternately 20 µl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of C_{22}H_{24}N_{2}O_{8}, taking into account the declared content of C_{22}H_{24}N_{2}O_{8},HCl in doxycycline hyclate RS.

Impurities The impurities limited by the requirements of this monograph include …

Note from the Secretariat: Impurities to be listed (with chemical names and structures).