DOXYCYCLINE HYCLATE TABLETS

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/06.184**  
*Revision of International Pharmacopoeia monograph on Doxycycline hyclate tablets*

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DOXYCYCLINE HYCLATE TABLETS:
Draft proposal for revision of published monograph in
*The International Pharmacopoeia*
(October 2006)

Proposal for revision of published monograph

**Doxycyclini hyclatis compressi - Doxycycline hyclate tablets**

**Category.** Antibacterial.

**Storage.** Doxycycline hyclate tablets should be kept in a tightly closed container.

**Labelling.** The designation on the container of doxycycline hyclate tablets should state that the active ingredient is in the hyclate form, and the quantity should be indicated in terms of the equivalent amount of doxycycline (C_{22}H_{24}N_{2}O_{8}).

**Additional information.** Strength in the current WHO Model list of essential medicines: 100mg of doxycycline (as hyclate).

**Requirements**

Comply with the monograph for "Tablets".

**Definition.** Doxycycline hyclate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of doxycycline, C_{22}H_{24}N_{2}O_{8}, stated on the label, if Assay method A is applied.

**Identity tests**

- Either tests A and D or tests B, C, and D may be applied.

**A.** Carry out the test as described under [1.14.1 Thin-layer chromatography](#), using silica gel R3 as the coating substance. Adjust the pH of a solution of 0.1g of disodium edetate R per ml to 9.0 with sodium hydroxide (~400g/l) TS, and spray this evenly onto the plate. Allow the plate to dry in a horizontal position for not less than 1 hour. Just before use,
Dry the plate in an oven at 110°C for 1 hour. Use a mixture of 59 volumes of dichloromethane R, 35 volumes of methanol R, and 6 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of the following 3 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 5mg of Doxycycline hyclate with 5ml of methanol R, filter, dilute the filtrate to 10ml with the same solvent, and use the resulting solution. For solution (B) dissolve 5mg of doxycycline hyclate RS in methanol R and dilute to 10ml with the same solvent. For solution (C) dissolve 5mg of doxycycline hyclate RS and 5mg of tetracycline hydrochloride RS in methanol R, and dilute to 10ml with the same solvent. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and examine the chromatogram in ultraviolet light (365nm).

A. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is valid only if the chromatogram obtained with solution C shows two clearly separated spots. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 12 volumes of ethyl acetate R, 12 volumes of acetic acid glacial R, 8 volumes of methanol R, and 2 volumes of ammonia R as the mobile phase. Apply separately to the plate 2µl of each of the following 3 solutions. For solution (A) shake a quantity of the contents of the powdered tablets containing the equivalent of about 8 mg of doxycycline in 10 ml of methanol R, filter, and use the resulting solution. For solution (B) dissolve 10mg of doxycycline hyclate RS in methanol R and dilute to 10ml with the same solvent. For solution (C) dissolve 10 mg of tetracycline hydrochloride RS in 10 ml of solution (B). After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and examine the chromatogram in ultraviolet light (254nm).

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 two clearly separated spots.

B. To a quantity of the powdered tablets containing the equivalent to of about 25mg of Doxycycline hyclate add about 52ml of sulfuric acid (~1760g/l) TS; an intense yellow colour is produced.
To a quantity of the powdered tablets containing the equivalent to about 0.1 g of doxycycline hyclate add 10 ml of water, filter, and use the filtrate for the following tests.

C. To 2.0 ml of the filtrate add 1 drop of ferric chloride (25 g/l) TS; a dark red-brown colour is produced.

D. To 1.0 ml of the filtrate add 5 drops of silver nitrate (40 g/l) TS; a white, curdy precipitate is formed which dissolves in 1.0 ml of ammonia (~100 g/l) TS.

**Loss on drying.** Weigh accurately approximately 1 g of the powdered tablets and dry for 2 hours at 105°, the loss is not more than 85 mg/g of the initial quantity taken.

[Note from the Secretariat: While it is unusual to include a test for Loss on drying in a monograph for a dosage form, a test is included here because of the nature of the substance and the need to calculate certain test results with reference to the dried (i.e. water and ethanol-free) material.]

**Light-absorbing impurities.** To a quantity of the powdered tablets containing the equivalent of about 0.10 g of doxycycline hyclate add 10 ml of a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R, shake, and filter, discarding the first 2 ml of filtrate. Measure the absorbance of a 1-cm layer at 490 nm; the absorbance does not exceed 0.2, calculated with reference to the dried tablets.

**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 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.

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 (6) (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 (6) (0.5% with reference to doxycycline hyclate).

Assay

Either method A or method B may be applied.

A. Weigh and powder 20 tablets. 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 (1A) use a quantity of the powered tablets sufficient to produce a solution containing the equivalent of 0.780mg of doxycycline hyclate per ml. Solution (2B) contains 0.80mg of doxycycline hyclate RS per ml, solution (3C) 0.80mg of 6-epidoxycycline hydrochloride RS per ml, solution (4D) 0.80mg of metacycline hydrochloride RS per ml. For solution (5E) mix 4.0ml of solution (2B) with 1.5ml of solution (3C) and 1.0ml of solution (4D), and dilute to 25ml with hydrochloric acid (0.01mol/l) VS and for solution
(6F) mix 2.0ml of solution (3)C and 2.0ml of solution (4)D 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)E. The assay test is not valid unless the resolution between the first peak (metacycline) and the second peak (6-epidoxycycline) is greater not less than 1.25, and the resolution between the second peak and the third peak (doxycycline) is greater not less 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 not more than 1.25. If necessary adjust the integrator parameters.

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

Measure the areas of the peak responses obtained in the chromatograms from solutions (1)A and (2)B, and calculate the percentage content of C22H24N2O8 in the tablets, taking into account the declared content of C22H24N2O8 in doxycycline hyclate RS.

B. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 50mg, accurately weighed, add 50ml of dimethylformamide R and shake for 1 hour. Centrifuge, and carry out the assay with the supernatant liquid as described under 3.1 Microbiological assay of antibiotics, using Bacillus cereus (NCTC 10320 or ATCC 11778) as the test organism, culture medium Cm10 with a final pH of 6.6, potassium dihydrogen phosphate (13.6g/l) TS as the buffer, an appropriate concentration of Doxycycline (usually between 0.2 and 2.0IU 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%. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the content stated on the label expressed in mg, with 870IU being equivalent to 1mg of doxycycline.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.
Impurities The impurities limited by the requirements of this monograph include those listed in the monograph for Doxycycline hyclate.

Note from the Secretariat: Impurities to be listed (with chemical names and structures) during revision of monograph for doxycycline hyclate are 6-epidoxycycline, metacycline, 4-epidoxycycline, 4-epi-6-epidoxycycline, oxytetracycline and 2-acetyl-2-decarbamoyldoxycycline.