INTERNATIONAL PHARMACOPOEIA MONOGRAPH ON DOXYCYCLINE HYCLATE CAPSULES

DRAFT FOR DISCUSSION

This document was provided by Professor Jin Shaohong, of the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, People's Republic of China. Comments have also been added by the WHO Secretariat. Depending on the final text the related monographs for Doxycycline hyclate and Doxycycline hyclate tablets will need to be subsequently revised.

Please address any comments you may have on this draft monograph, by 18 August 2006, to Dr S. Kopp, Quality Assurance and Safety: Medicines, Medicines Policy and Standards, World Health Organization, 1211 Geneva 27, Switzerland, fax: (+41 22) 791 4730 or e-mail: kopps@who.int, with a copy to rabouhansm@who.int.

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Please send any request for permission to:

Dr Sabine Kopp, Quality Assurance & Safety: Medicines (QSM), Department of Medicines Policy and Standards (PSM), World Health Organization, CH-1211 Geneva 27, Switzerland.
Fax: (41-22) 791 4730; e-mails: kopps@who.int; bonnyw@who.int

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*International Pharmacopoeia monograph on doxycycline hyclate capsules*

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation of first draft by WHO Collaborating Centre for Drug Quality Assurance, NICPBP, Beijing, China</td>
<td>May-June 2006</td>
</tr>
<tr>
<td>Circulation of first draft for comments</td>
<td>July 2006</td>
</tr>
<tr>
<td>Discussion of first draft + any comments received during consultation on specifications for medicines and quality control laboratory issues</td>
<td>25-27 July 2006</td>
</tr>
<tr>
<td>Circulation of second draft for comments</td>
<td>August/September 2006</td>
</tr>
<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
<td>16-20 October 2006</td>
</tr>
<tr>
<td>Eventual circulation of third draft, depending on Expert Committee's decision</td>
<td>November 2006</td>
</tr>
</tbody>
</table>
DOXYCYCLINE HYCLATE CAPSULES:

Draft proposal for The International Pharmacopoeia

(September 2006)

DOXYCYCLIN HYCLATIS CAPSULAE
DOXYCYCLINE HYCLATE CAPSULES

Category. Antibacterial drug.

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

[Note from the Secretariat: The Ph. Int. monograph for the API defines doxycycline hyclate as doxycycline monohydrochloride, compound with ethanol (2:1), monohydrate with a relative molecular mass of 1026. The content declaration (strength) on the label is, however, in terms of the active moiety - doxycycline (C$_{22}$H$_{24}$N$_{2}$O$_{8}$) with a relative molecular mass of approximately 444.9. The content limits in the Definition are therefore expressed in terms of doxycycline as are the amounts of material required for testing.]

Labelling. The designation on the container of doxycycline hyclate capsules should state that the active ingredient is in the hyclate form, and the quantity should be indicated in terms of the equivalent amount of anhydrous doxycycline (C$_{22}$H$_{24}$N$_{2}$O$_{8}$).

Additional information. Strength in the current WHO Model List of Essential Medicines: the equivalent of 100mg of anhydrous doxycycline (as hyclate).

Requirements
Comply with the monograph for “Capsules”.

Definition. Doxycycline hyclate capsules contain Doxycycline hyclate. They contain not less than 90.0% and not more than 110.0% of the amount of anhydrous doxycycline, C$_{22}$H$_{24}$N$_{2}$O$_{8}$, stated on the label.

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 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 32 solutions. For solution (A) shake a quantity of the contents of the capsules containing the equivalent of about 84 mg of anhydrous doxycycline in 10 ml of methanol R, filter, dilute the filtrate to 10ml with the same solvent, 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 contents of the capsules containing the equivalent of about 24 mg of anhydrous doxycycline, add about 5ml of sulfuric acid (~1760g/l) TS; an intense yellow colour is produced.

C. To a quantity of the contents of the capsules containing the equivalent of about 0.105 g of anhydrous doxycycline, add 10 ml of water R and filter. Reserve part of the filtrate for test D. To 2.0ml of the filtrate add 1 drop of ferric chloride (25g/l) TS; a dark red-brown colour is produced.

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

Loss on drying. Weigh accurately approximately 1 g of the contents of the capsules and dry to constant weight for 2 hours at 105 °C, the loss is not more than 85mg/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 possible need to calculate certain test results with reference to the dried (i.e. anhydrous water and ethanol-free) material.]

Related substances/Light-absorbing impurities

[Note from the Secretariat: The possibility of including (1) a liquid chromatographic test for Related substances (as in the monograph for Doxycycline hyclate) and a test for Light-absorbing impurities (as in the monographs for Doxycycline hyclate and
Doxycycline hyclate tablets) is under investigation.

**Light-absorbing impurities.** Dissolve the contents of five capsules as completely as possible in sufficient of a mixture of 1 volume of hydrochloric acid (1mol/l) VS and 99 volumes of methanol R to produce a solution containing the equivalent of 10 mg per ml of doxycycline. Shake and filter, discarding the first 2 ml of filtrate. Measure the absorbance of a 1-cm layer of the filtrate at 490nm; the absorbance does not exceed 0.2, calculated with reference to the dried capsule content.

**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 (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.** 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 (8-10µm). As the mobile phase, use a solution prepared as follows: transfer 60.0g of tert-butanol R with the aid of 200ml water R 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 R.

Prepare the following solutions in hydrochloric acid (0.01mol/l) VS. For solution (1A) use an amount of the mixed contents of 20 the capsules to produce a solution containing the equivalent of 0.70mg of anhydrous doxycycline per ml. Solution (2B) contains 0.80mg of doxycycline hyclate RS per ml. Solution (3C) 0.80mg of
6-epidoxycycline hydrochloride RS per ml and solution (4) 0.80mg of metacycline hydrochloride RS per ml. For solution (5) mix 4.0ml of solution (2)B with 1.5ml of solution (3)E and 1.0ml of solution (4)D, 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 20µl 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 not less greater 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 20µl each of solutions (1)A and (2)B.

Measure the areas of the peak responses in the chromatograms obtained with solution (1)A and (2)B, and calculate the percentage content of C_{22}H_{24}N_{2}O_{8} in the capsules, taking into account the declared content of C_{22}H_{24}N_{2}O_{8} in doxycycline hyclate RS.

**Dissolution test** [to be added?]

**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.

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