**TYLOSIN FOR VETERINARY USE**

Tylosinum ad usum veterinarium

**DEFINITION**

Tylosin for veterinary use is a mixture of macrolide antibiotics produced by a strain of *Streptomyces fradiae* or by any other means. The main component of the mixture is (4R,5S, 6S,7R,9R,11E,13E,15R,16R,15S)-[[6-deoxy-2,3-di-O-methyl-β-D-allopyranosyl]oxy][methyl]-6-[[3,6-dIDEOXY-3-C-methyl-6,7-D-ribopyranosyl]-3-dimethylamino]-β-D-glucopyranosyl[oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-o xoethyl)oxacyclohexadeca-11,13-diene-2,10-dione (tylosin A, tylosin B, tylosin C and tylosin D is not less than 95.0 per cent).

**CHARACTERS**

An almost white or slightly yellow powder, slightly soluble in water, freely soluble in ethanol and in methylene chloride. It dissolves in dilute solutions of mineral acids.

**IDENTIFICATION**

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with tylosin CRS.

B. Examine the chromatograms obtained in the test for composition. The retention time and size of the principal peak in the chromatogram obtained with the test solution are the same as those of the principal peak in the chromatogram obtained with reference solution (a).

C. Dissolve about 30 mg in a mixture of 0.15 ml of water R, 2.5 ml of acetic anhydride R and 7.5 ml of pyridine R. Allow to stand for about 10 min. No green colour develops.

**TESTS**

**pH** (2.2.3). Suspend 0.25 g in 10 ml of carbon dioxide-free water R. The pH of the suspension is 8.5 to 10.5.

**COMPOSITION.** Examine by liquid chromatography (2.2.29). Prepare the solutions immediately before use.

The content of tylosin A is not less than 80.0 per cent and the sum of the contents of tylosin A, tylosin B, tylosin C and tylosin D is not less than 95.0 per cent.

**Test solution.** Dissolve 20.0 mg of the substance to be examined in a mixture of equal volumes of acetonitrile R and water R and dilute to 100.0 ml with the same mixture of solvents.

**Reference solution (a).** Dissolve 20.0 mg of tylosin CRS in a mixture of equal volumes of acetonitrile R and water R and dilute to 10 ml with the same mixture of solvents.

**Reference solution (b).** Dissolve 2 mg of tylosin CRS and 2 mg of tylosin D CRS in a mixture of equal volumes of acetonitrile R and water R and dilute to 10 ml with the same mixture of solvents.

The chromatographic procedure may be carried out using:

- a stainless steel column 0.20 m long and 4.6 mm in internal diameter packed with octadecylsilyle silica gel for chromatography (5 µm),
- as mobile phase at a flow rate of 1.0 ml/min a mixture of 40 volumes of acetonitrile R and 60 volumes of a 200 g/l solution of sodium perchlorate R previously adjusted to pH 2.5 using 1 M hydrochloric acid,
- as detector a spectrophotometer set at 290 nm, maintaining the temperature of the column at 35 °C.

Inject 20 µl of reference solution (b). When the chromatograms are recorded in the prescribed conditions, the retention time of tylosin A is about 12 min. The test is not valid unless, in the chromatogram obtained, the resolution between the peaks corresponding to tylosin A and tylosin D is at least 2.0. Inject 20 µl of the test solution and 20 µl of reference solution (a). Calculate the percentage content of the constituents from the areas of the peaks in the chromatogram obtained with the test solution by the normalisation procedure.

**Tyramine.** In a 25.0 ml volumetric flask, dissolve 50.0 mg of the substance to be examined in 5.0 ml of a 3.4 g/l solution of phosphoric acid R. Add 1.0 ml of pyridine R and 2.0 ml of a saturated solution of ninhydrin R (about 40 g/l). Close the flask with a piece of aluminium foil and heat in a water-bath at 85 °C for 30 min. Cool the solution rapidly and dilute to 25.0 ml with water R. Mix and measure immediately the absorbance (2.2.25) of the solution at 570 nm using a blank solution as the compensation liquid. The absorbance is not greater than that of a standard prepared at the same time and in the same manner using 5.0 ml of a 35 mg/l solution of tyramine R in a 3.4 g/l solution of phosphoric acid R (0.35 per cent). If intended for use in the manufacture of parenteral dosage forms, the absorbance is not greater than that of a standard prepared at the same time and in the same manner using 5.0 ml of a 15 mg/l solution of tyramine R in a 3.4 g/l solution of phosphoric acid R (0.15 per cent).

**Loss on drying** (2.2.32). Not more than 5.0 per cent, determined on 1,000 g by drying in an oven at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

**Sulphated ash** (2.4.14). Not more than 3.0 per cent, determined on 1.0 g.

**ASSAY**

Carry out the microbiological assay of antibiotics (2.7.2). Use tylosin CRS as the reference substance.

**STORAGE**

Store protected from light.
DEFINITION

Solution of the dihydrogen phosphate of a mixture of macrolide antibiotics produced by a strain of *Streptomyces fradiae* or by any other means.

The main component is the phosphate of \((4R,5S,6S,7R,9R,11E,13E,15R,16R)-15-\{[(6-deoxy-2,3-di-O-methyl-[\beta]-allopregn-3-ol)-\(\alpha\)-ribohexopyranosyl]-3-[\(\alpha\)-\(D\)-glucopyranosyl](\(\alpha\)-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-[\(\beta\)-oxoethyl]oxacyclohexadeca-11,13-diene-2,10-dione (tylosin A phosphate)\}

<table>
<thead>
<tr>
<th>Tylosin</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Mol. Formula</th>
<th>M_r</th>
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<td>A</td>
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<td>OCH_3</td>
<td>CHO</td>
<td>C_{46}H_{67}N_{17}O_{17}</td>
<td>916</td>
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<tr>
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<td>H</td>
<td>OCH_3</td>
<td>CHO</td>
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<tr>
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<td>CHO</td>
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<tr>
<td>D</td>
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<td>CH_2OH</td>
<td>C_{46}H_{67}N_{17}O_{17}</td>
<td>918</td>
</tr>
</tbody>
</table>

The solution also contains sodium dihydrogen phosphate.

Potency: minimum 800 IU/mg of dry residue. Tylosins A, B, C and D contribute to the potency.

CHARACTERS

Appearance: yellow or brownish-yellow, viscous liquid.

Solubility: miscible with water.

IDENTIFICATION

A. Dilute an amount of the preparation to be examined equivalent to 400 000 IU of tylosin phosphate to 100.0 ml with water.

B. Examine the chromatograms obtained in the test for composition.

Result: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Dilute an amount of the preparation to be examined equivalent to 400 000 IU of tylosin phosphate in 10 ml of water.

TESTS

pH: 5.5 to 6.5.

Composition. Liquid chromatography: use the normalisation procedure.

Prepare the solutions immediately before use.

Test solution. Dilute an amount of the preparation to be examined equivalent to 50 000 IU of tylosin phosphate to 200 ml with a mixture of equal volumes of acetonitrile and water.

Reference solution (a). Dissolve 20 mg of tylosin CRS in a mixture of equal volumes of acetonitrile and water and dilute to 100 ml with the same mixture of solvents.

Reference solution (b). Dissolve 2 mg of tylosin CRS and 2 mg of tylosin D CRS in a mixture of equal volumes of acetonitrile and water and dilute to 10 ml with the same mixture of solvents.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of acetonitrile and water. Dilute 1.0 ml of this solution to 10.0 ml with the same mixture of solvents.

Column:

- size: \(l = 0.20\) m, \(d = 4.6\) mm;
- stationary phase: octadecylsilyle silicic gel for chromatography (5 µm);
- temperature: 35 °C.

Mobile phase: mix 40 volumes of acetonitrile and 60 volumes of a 200 g/l solution of sodium perchlorate previously adjusted to pH 2.5 using a 36.5 g/l solution of hydrochloric acid.