Tylosin Injection

General Notices

Details for the public consultation of this monograph are as follows:

<table>
<thead>
<tr>
<th>EAG ABS</th>
<th>Antibiotics</th>
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<tbody>
<tr>
<td>Contact Details</td>
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<tr>
<td>Deadline for Comment</td>
<td>30th June 2020</td>
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<tr>
<td>Target Publication (subject to change)</td>
<td>BP 2022</td>
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</tbody>
</table>

Notes:
- Revision to Identification Composition and Related substances
- Identification test B removed as IR is sufficiently discriminatory.
- Comp + RS: Liquid Chromatography method has been updated.
- Impurities limits been tightened to reflect current analytical capability and VICH requirement.
- If limits are too restrictive, please provide batch/stability data to demonstrate that an increase is required.

Action and use

Macrolide antibacterial.

DEFINITION

Tylosin Injection is a sterile solution of Tylosin in a mixture of equal parts by volume of Propylene Glycol and Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

CHARACTERISTICS

A pale yellow to amber-coloured solution.

IDENTIFICATION

A. Dilute a volume containing 0.1 g of Tylosin with water to give a solution containing 0.02% w/v of Tylosin. To 5 mL of this solution add 10 mL of 0.1M sodium hydroxide and extract with 10 mL of dichloromethane. Separate the chloroform layer and extract it with 25 mL of 0.1M hydrochloric acid. Discard the chloroform, wash the aqueous layer
with 3 mL of dichloromethane, discard the washings and filter. The light absorption of the filtrate, Appendix II B, in the range 230 to 350 nm, exhibits a maximum only at 290 nm. The absorbance at the maximum is about 0.94.

**TESTS**

**Composition**

Carry out the method for liquid chromatography, Appendix III D, using the normalisation procedure. Use the following solutions, prepared immediately before use.

**Solution A** 2.582% w/v solution of potassium dihydrogen orthophosphate adjusted to pH 5.5 using a 1.32% w/v solution of dipotassium hydrogen orthophosphate.

(1) Dilute the injection with 20% v/v acetonitrile in water to produce a solution containing the equivalent of 0.1% w/v of tylosin.

(2) 0.1% w/v of tylosin for system suitability EPCRS in 20% v/v acetonitrile in water.

**CHROMATOGRAPHIC CONDITIONS**

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with end-capped octadecylsilyl silica gel for chromatography (5 µm) (Nucleosil C18 is suitable).

(b) Use gradient elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use an a column temperature of 60 °.

(e) Use a detection wavelength of 280 nm.

(f) Inject 20 µL of each solution.

**MOBILE PHASE**

**Mobile phase A** 100 volumes of solution A, 275 volumes of acetonitrile and 625 volumes of water.

**Mobile phase B** 100 volumes of solution A, 500 volumes of acetonitrile and 400 volumes of water.

<table>
<thead>
<tr>
<th>Time (Minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-25</td>
<td>100</td>
<td>0</td>
<td>isocratic</td>
</tr>
<tr>
<td>25-45</td>
<td>100→84</td>
<td>0→16</td>
<td>linear gradient</td>
</tr>
<tr>
<td>45-65</td>
<td>84</td>
<td>16</td>
<td>isocratic</td>
</tr>
<tr>
<td>65-70</td>
<td>84→44</td>
<td>16→56</td>
<td>linear gradient</td>
</tr>
<tr>
<td>70-82</td>
<td>44</td>
<td>56</td>
<td>isocratic</td>
</tr>
<tr>
<td>82-83</td>
<td>44→100</td>
<td>56→0</td>
<td>linear gradient</td>
</tr>
<tr>
<td>83-90</td>
<td>100</td>
<td>0</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to tylosin (retention time about 65 minutes) are: impurity E, about 0.23; tylosin B, about 0.31; impurity A, about 0.38; tylosin C, about 0.60; tylosin D, about 0.78; impurity N, about 0.81, impurity O, about 0.85, impurity R, about 1.17 and impurity S, about 1.20.
SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the resolution between tylosin B and impurity A is at least 2.0;

the resolution between the peaks due to impurities N and O is at least 1.5 and;

the resolution between the peaks due to impurities R and S is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (2), identify the peaks due to Tylosin A, B, C and D. Integrate all peaks at any area greater than 0.1% to determine the total peak area. Calculate the percentage content of each of the components by normalisation. In the chromatogram obtained with solution (1):

the content of tylosin A is not less than 80%;

the total content of tylosins A, B, C and D is not less than 90%.

Disregard any peaks with an area less than 0.10%.

Related substances

Carry out the method for liquid chromatography, Appendix III D, using the normalisation procedure. Use the following solutions, prepared immediately before use.

(1) Dilute the injection with 20% v/v acetonitrile in water to produce a solution containing the equivalent of 0.1% w/v of tylosin.

(2) 0.1% w/v of tylosin for system suitability EPCRS in 20% v/v acetonitrile in water.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions under Composition may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the resolution between tylosin B and impurity A is at least 2.0;

the resolution between the peaks due to impurities N and O is at least 1.5 and;

the resolution between the peaks due to impurities R and S is at least 1.3.

LIMITS

Using the chromatogram obtained with solution (2), identify the peaks due to impurities A, N, O, E, R and S, and tylosin C. In the chromatogram obtained with solution (1), integrate all peaks at any area greater than 0.1% to determine the total peak area. Calculate the percentage of each impurity by normalisation:

the area of any peak due to impurity A is not greater than 2.0%;

the sum of the areas of the peaks eluting between the peak due to impurity A and the peak due to tylosin C is not greater than 2.0%;

the area of any peak due to impurities N, O, E, R or S is not greater than 1.0%;
the area of any other secondary peak is not greater than 1.0%;

the sum of the areas of any secondary peak is not greater than 5.0%.

Disregard any peaks due to tylosin B and tylosin D.

Tyramine

Dilute a volume containing 0.1 g of Tylosin with 5 mL of 0.03M orthophosphoric acid in a 25 mL graduated flask, add 1 mL of pyridine and 2 mL of a saturated solution of ninhydrin (approximately 4% w/v). Close the flask by covering with a piece of aluminium foil and heat in a water bath at 85° for at least 20 minutes. Cool rapidly and add sufficient water to produce 25 mL. Measure the absorbance of the resulting solution without delay at 570 nm, Appendix II B, using in the reference cell a solution prepared in the same manner but omitting the injection being examined. The absorbance is not greater than that obtained by carrying out the procedure at the same time using 5 mL of a solution in 0.03M orthophosphoric acid containing 35 µg of tyramine per mL and beginning at the words ‘add 1 mL…’ (0.175%).

ASSAY

Carry out the microbiological assay of antibiotics, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of tylosin in the injection taking each 1000 IU found to be equivalent to 1 mg of tylosin. 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 stated content.

STORAGE

Tylosin Injection should be kept in a cool place.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Tylosin.