Edition: BP 2025 (Ph. Eur. 11.6 update)

Tylosin Premix

General Notices

Action and use

Macrolide antibacterial

DEFINITION

Tylosin Premix contains Tylosin Phosphate.

The premix complies with the requirements stated under Premixes and with the following requirements.

CHARACTERS

Light brown granules.

IDENTIFICATION

A. In the Composition, record the UV spectrum of the principal peak in the chromatograms obtained with solutions (1) and (2) with a diode array detector in the range of 210 to 400 nm.

The UV spectrum of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2);

the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the peak in the chromatogram obtained with solution (2).

B. Shake 2 g of the premix with 20 mL of *water* and filter. The filtrate, after neutralisation, if necessary, yields reaction B characteristic of *phosphates*, Appendix VI.

TESTS

Composition

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

Solution A Dissolve 25.82 g of *potassium dihydrogen orthophosphate* in 800 mL, adjust to pH 5.5 using a 1.32% w/v solution of *dipotassium hydrogen orthophosphate* and dilute to 1 L.

(1) Dissolve a quantity of the premix in 20% v/v *acetonitrile* and dilute with the same solvent to produce a solution containing the equivalent of 0.1% w/v of tylosin. Filter through a 0.45-µm filter.
(2) 0.1% w/v of *tylosin for system suitability EPCRS* in 20% v/v *acetonitrile*.
(3) Dilute 1 volume of solution (1) to 100 volumes with *water*. Dilute 1 volume of the resulting solution to 10 volumes.

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 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 for chromatography*.

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

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-45	100→84	0→16	linear gradient
45-65	84	16	isocratic
65-70	84→44	16→56	linear gradient
70-82	44	56	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to tylosin A (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.

Use the chromatogram obtained with solution (2) to identify peaks due to tylosin A, B, C and D and impurities A, E, N, O, R and S.

SYSTEM SUITABILITY

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

the *resolution* between the peaks due to 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

In the chromatogram obtained with solution (1), integrate all peaks present with an area greater than the area of the principal peak in the chromatogram obtained with solution (3) to determine the total peak area. Calculate the percentage content of each of the components by *normalisation*:

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

the total content of tylosins A, B, C and D is not less than 95%.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, as described in the test for Composition.

LIMITS

In the chromatogram obtained with solution (1):

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

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

the area of any peak due to impurity N, O, E, R or S is not greater than 1.0% by *normalisation* (for each);

the area of any other *secondary peak* is not greater than 1.0% by *normalisation*;

the sum of the areas of any *secondary peaks* is not greater than 5.0% by *normalisation*.

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 premix taking each 1000 IU found to be equivalent to 1 mg of tylosin. The upper fiducial limit of error is not less than 90.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

IMPURITIES

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