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

Tylosin Injection

General Notices

Action and use

Macrolide antibacterial.

DEFINITION

Tylosin Injection is a sterile solution of Tylosin.

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 produce 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 dichloromethane layer and extract it with 25 mL of 0.1M *hydrochloric acid*. Discard the dichloromethane layer, 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.
B. To 10 mL of the filtrate obtained in test A add 1 mL of 2M *sodium hydroxide*, heat on a water bath for 20 minutes and cool. The *light absorption*, Appendix II B, in the range 250 to 430 nm, exhibits a maximum at 332 nm.

TESTS

Composition

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

(1) Dilute the injection with 20% v/v of *acetonitrile* to produce a solution containing 0.1% w/v of Tylosin.
(2) 0.1% w/v of *tylosin for system suitability EPCRS* in *acetonitrile (20%)*.
(3) Dilute 1 volume of solution (1) to 100 volumes with *water*. Dilute 1 volume of the resulting solution to 10 volumes with *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 a column temperature of 60°.
(e) Use a detection wavelength of 280 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

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

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, 400 volumes of *water* 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 0.2; tylosin B, about 0.31; impurity A, about 0.38; tylosin C, about 0.6; tylosin D, about 0.78; impurity N, about 0.81; impurity O, about 0.9; 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 90%.

Related substances

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

LIMITS

In the chromatogram obtained with solution (1):

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

the area of any peak due to impurity B is not greater than 5.0%;

the area of any peak due to impurity D is not greater than 1.5%;

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

the sum of the areas of any *secondary peaks* is not greater than 10.0%.

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 90.0% and the lower fiducial limit of error is not more than 112.4% of the stated content.

STORAGE

Tylosin Injection should be kept in the original container, protected from light and stored below 30°.

IMPURITIES

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