Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.

Top of the plate	
———	———
β-Pinene: a pink zone	A pink zone (β-pinene)
	A pink zone
———	———
Linalol: a pinkish-grey zone	
	3 faint violet zones
	A faint yellow zone
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results: the peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS

Relative density (*2.2.5*): 0.856 to 0.872.

Refractive index (*2.2.6*): 1.465 to 1.475.

Optical rotation (*2.2.7*): – 40° to – 28°.

Acid value (*2.5.1*): maximum 1.0.

Peroxide value (*2.5.5, Method B*): maximum 20.

Fatty oils and resinified essential oils (*2.8.7*). It complies with the test for fatty oils and resinified essential oils.

Chromatographic profile. Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution. The substance to be examined.

Reference solution (a). Dissolve 30 µl of *α-pinene R*, 10 mg of *camphene R*, 20 µl of *β-pinene R*, 10 µl of *car-3-ene R*, 10 µl of *β-myrcene R*, 20 µl of *limonene R*, 10 µl of *longifolene R*, 10 µl of *β-caryophyllene R* and 10 mg of *caryophyllene oxide R* in 1 ml of *hexane R*.

Reference solution (b). Dissolve 5 µl of *β-caryophyllene R* in *hexane R* and dilute to 1 ml with the same solvent. Dilute 0.1 ml to 1 ml with *hexane R*.

Column:

– *material*: fused silica;
– *size*: *l* = 60 m, Ø = 0.25 mm;
– *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1.0 ml/min.

Split ratio: 1:63.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 80	60 → 200
	80 - 120	200
Injection port		200
Detector		250

Detection: flame ionisation.

Injection: 0.5 µl.

Elution order: order indicated in the composition of the reference solution (a); record the retention times of these substances.

System suitability:

– *resolution*: minimum 1.5 between the peaks due to car-3-ene and β-myrcene in the chromatogram obtained with reference solution (a).

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of reference solution (a) in the chromatogram obtained with the test solution.

Determine the percentage content of these components. The limits are within the following ranges:

– *α-pinene*: 70.0 per cent to 85.0 per cent;
– *camphene*: 0.5 per cent to 1.5 per cent;
– *β-pinene*: 11.0 per cent to 20.0 per cent;
– *car-3-ene*: maximum 1.0 per cent;
– *β-myrcene*: 0.4 per cent to 1.5 per cent;
– *limonene*: 1.0 per cent to 7.0 per cent;
– *longifolene*: 0.2 per cent to 2.5 per cent;
– *β-caryophyllene*: 0.1 per cent to 3.0 per cent;
– *caryophyllene oxide*: maximum 1.0 per cent;
– *disregard limit*: area of the peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Residue on evaporation (*2.8.9*): maximum 2.5 per cent.

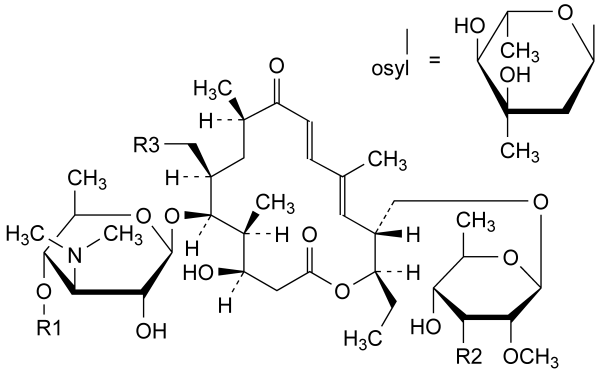
STORAGE

In a well-filled, airtight container, protected from light and at a temperature not exceeding 25 °C.

01/2008:1273

TYLOSIN FOR VETERINARY USE

Tylosinum ad usum veterinarium



Name	Mol. Formula	R1	R2	R3
tylosin A	$C_{46}H_{77}NO_{17}$	osyl	OCH_3	CHO
tylosin B	$C_{39}H_{65}NO_{14}$	H	OCH_3	CHO
tylosin C	$C_{45}H_{75}NO_{17}$	osyl	OH	CHO
tylosin D	$C_{46}H_{79}NO_{17}$	osyl	OCH_3	CH_2OH

DEFINITION

Mixture of macrolide antibiotics produced by a strain of *Streptomyces fradiae* or by any other means. The main component of the mixture is (4*R*,5*S*,6*S*,7*R*,9*R*,11*E*,13*E*,15*R*,16*R*)-15-[[(6-deoxy-2,3-di-*O*-methyl-β-D-allopyranosyl)oxy]methyl]-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-

Monographs T-Z

glucopyranosyl]oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-diene-2,10-dione (tylosin A, M_r 916). Tylosin B (desmycosin, M_r 772), tylosin C (macrocin, M_r 902) and tylosin D (relomycin, M_r 918) may also be present. They contribute to the potency of the substance to be examined.

Potency: minimum 900 IU/mg (dried substance).

CHARACTERS

Appearance: almost white or slightly yellow powder.

Solubility: slightly soluble in water, freely soluble in anhydrous ethanol and in methylene chloride. It dissolves in dilute solutions of mineral acids.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *tylosin CRS*.

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

Results: 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. 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*): 8.5 to 10.5.

Suspend 0.25 g in 10 ml of *carbon dioxide-free water R*.

Composition. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Prepare the solutions immediately before use.*

Solvent mixture: *acetonitrile R, water R* (50:50 *V/V*).

Test solution. Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). Dissolve 2 mg of *tylosin phosphate for peak identification CRS* (containing tylosins A, B, C and D) in the solvent mixture and dilute to 10 ml with the solvent mixture.

Reference solution (b). Dissolve 2 mg of *tylosin CRS* and 2 mg of *tylosin D CRS* in the solvent mixture and dilute to 10 ml with the solvent mixture.

Column:

- *size*: *l* = 0.20 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase: mix 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*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 290 nm.

Injection: 20 µl.

Retention time: tylosin A = about 12 min.

Identification of peaks: use the chromatogram supplied with *tylosin phosphate for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to tylosins A, B, C and D.

System suitability: reference solution (b):

- *resolution*: minimum 2.0 between the peaks due to tylosins A and D.

Limits:

- *tylosin A*: minimum 80.0 per cent;
- *sum of tylosins A, B, C and D*: minimum 95.0 per cent.

Tyramine: maximum 0.35 per cent and maximum 0.15 per cent, if intended for use in the manufacture of parenteral dosage forms.

In a 25.0 ml volumetric flask, dissolve 50.0 mg 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*. 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*.

Loss on drying (*2.2.32*): maximum 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*): maximum 3.0 per cent, determined on 1.0 g.

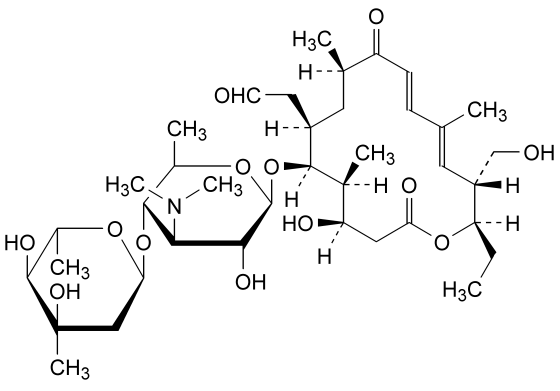
ASSAY

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

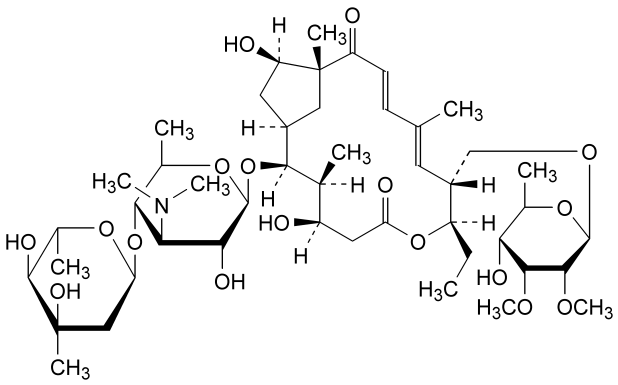
STORAGE

Protected from light.

IMPURITIES



A. desmycinosyltylosin,

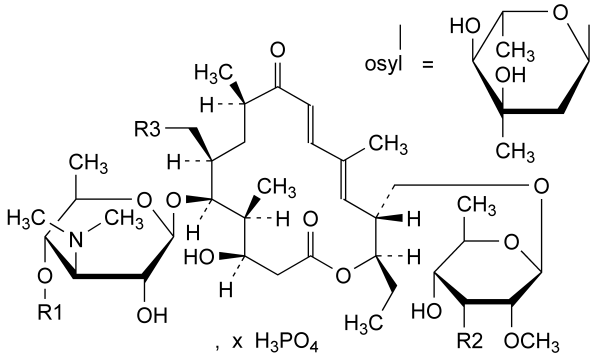


B. tylosin A aldol.

01/2008:1661

TYLOSIN PHOSPHATE BULK SOLUTION FOR VETERINARY USE

Tylosini phosphatis solutio ad usum veterinarium



Tylosin	R1	R2	R3	Mol. Formula	M_r
A	osyl	OCH_3	CHO	$C_{46}H_{77}NO_{17}$	916
B	H	OCH_3	CHO	$C_{39}H_{65}NO_{14}$	772
C	osyl	OH	CHO	$C_{45}H_{75}NO_{17}$	902
D	osyl	OCH_3	CH_2OH	$C_{46}H_{79}NO_{17}$	918

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 (4*R*,5*S*,6*S*,7*R*,9*R*,11*E*,13*E*,15*R*,16*R*)-15-[[(6-deoxy-2,3-di-*O*-methyl-β-D-allopyranosyl)oxy]methyl]-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-diene-2,10-dione (tylosin A phosphate). The phosphates of tylosin B (desmycosin phosphate), tylosin C (macrocin phosphate) and tylosin D (relomycin phosphate) may also be present. The solution also contains sodium dihydrogen phosphate.

Potency: minimum 800 IU per milligram 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. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dilute an amount of the preparation to be examined equivalent to 400 000 IU of tylosin phosphate to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 100.0 ml with *water R*.

Spectral range: 230-350 nm.

Absorption maximum: at 290 nm.

Absorbance at the absorption maximum: minimum 0.70.

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

Results: 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 R*. The solution gives reaction (a) of phosphates (*2.3.1*).

TESTS

pH (*2.2.3*): 5.5 to 6.5.

Dilute 1.0 g in 10 ml of *carbon dioxide-free water R*.

Composition. Liquid chromatography (*2.2.29*): 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 R* and *water R*.

Reference solution (a). Dissolve 2 mg of *tylosin phosphate for peak identification CRS* (containing tylosins A, B, C and D) 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.

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

Column:

– *size*: *l* = 0.20 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 μm);
– *temperature*: 35 °C.

Mobile phase: mix 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 a 36.5 g/l solution of *hydrochloric acid R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 290 nm.

Injection: 20 μl.

Run time: 1.8 times the retention time of tylosin A.

Identification of tylosins: use the chromatogram supplied with *tylosin phosphate for peak identification CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to tylosins A, B, C and D.