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 preparations.

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 preparations, 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.

Sulfated ash (*2.4.14*): maximum 3.0 per cent, determined on 1.0 g.

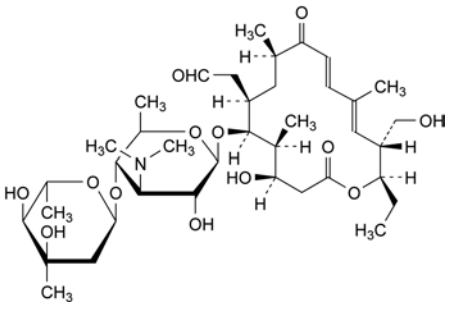
ASSAY

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

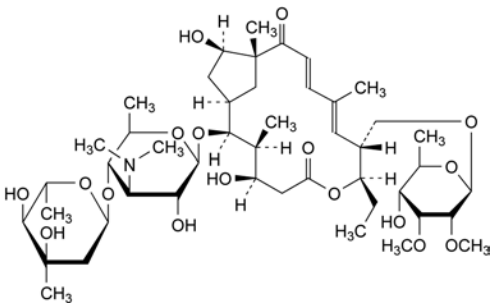
STORAGE

Protected from light.

IMPURITIES



A. desmycinosyltylosin,

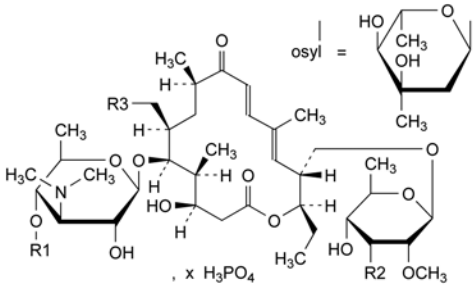


B. tylosin A aldol.

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.

Relative retention with reference to tylosin A (retention time = about 12 min): impurity A = about 0.35; tylosin C = about 0.5; tylosin B = about 0.6; tylosin D = about 0.85; impurity B = about 0.9.

System suitability: reference solution (b):

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

Limits:

- *tylosin A*: minimum 80.0 per cent;
- *sum of the contents of tylosin A, tylosin B, tylosin C and tylosin D*: minimum 95.0 per cent;
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c).

Tyramine. In a 25.0 mL volumetric flask, dissolve an amount of the preparation to be examined equivalent to 50 000 IU of tylosin phosphate 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 aluminium foil and heat in a water-bath at 85 °C for 20-30 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*.

Phosphate: 8.5 per cent to 10.0 per cent of PO_4, calculated with reference to the dry residue (see Assay).

Test solution. Dissolve an amount of the preparation to be examined equivalent to 200 000 IU of tylosin phosphate in 50 mL of *water R*. Add 5.0 mL of *dilute sulfuric acid R* and dilute to 100.0 mL with *water R*. To 2.0 mL of this solution add successively, mixing after each addition, 10.0 mL of *water R*, 5.0 mL of *ammonium molybdate reagent R2*, 1.0 mL of *hydroquinone solution R* and 1.0 mL of a 200 g/L solution of *sodium metabisulfite R*. Allow to stand for at least 20 min and dilute to 50.0 mL with *water R*. Mix thoroughly.

Reference solution (a). To 1.0 mL of a standard solution containing 0.430 g/L of *potassium dihydrogen phosphate R* (corresponds to 300 ppm of PO_4) add successively, mixing after each addition, 10.0 mL of *water R*, 5.0 mL of *ammonium molybdate reagent R2*, 1.0 mL of *hydroquinone solution R* and 1.0 mL of a 200 g/L solution of *sodium metabisulfite R*. Allow to stand for at least 20 min and dilute to 50.0 mL with *water R*. Mix thoroughly.

Reference solution (b). Prepare as reference solution (a) but using 2.0 mL of the standard solution.

Reference solution (c). Prepare as reference solution (a) but using 5.0 mL of the standard solution.

Compensation liquid. Prepare as reference solution (a) but omitting the standard solution.

Measure the absorbance (*2.2.25*) of the test solution and of the reference solutions at 650 nm. Draw a calibration curve with the absorbances of the 3 reference solutions as a function of the quantity of phosphate in the solutions and read from the curve the quantity of phosphate in the test solution. Determine the percentage content of PO_4, calculated with reference to the dry residue (see Assay).

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*).

Use *tylosin CRS* as the reference substance. Calculate the potency from the mass of the dry residue and the activity of the solution.

Dry residue. Dry 3.0 g of the preparation to be examined *in vacuo* at 60 °C for 3 h and weigh.

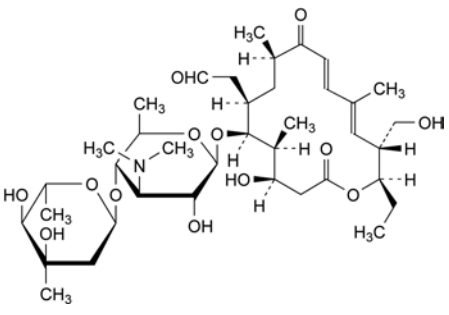
STORAGE

Protected from light, at a temperature of 2 °C to 8 °C.

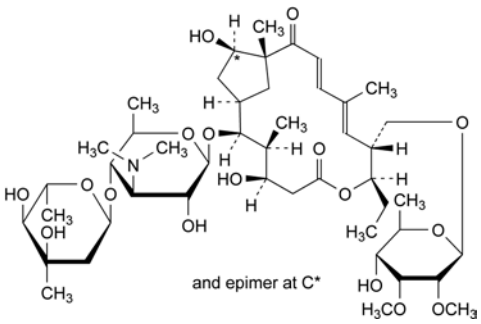
LABELLING

The label states the concentration of the solution in International Units per milligram of preparation.

IMPURITIES



A. desmycinosyltylosin A,

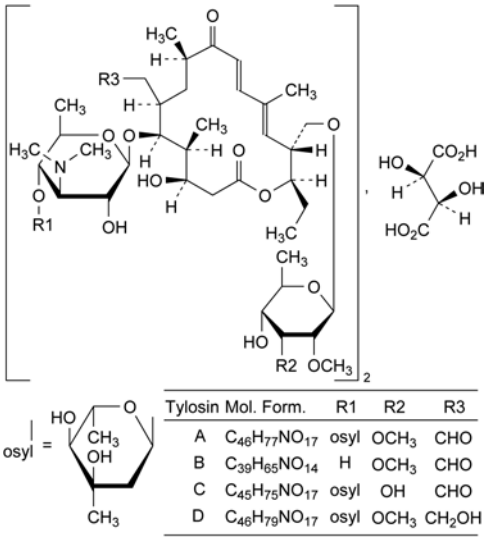


B. (1*R*,2*S*,3*S*,4*R*,8*R*,9*R*,10*E*,12*E*,15*R*,16*RS*)-9-[[(6-deoxy-2,3-di-*O*-methy-β-D-allopyranosyl)oxy]methyl]-2-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-8-ethyl-4,16-dihydroxy-3,11,15-trimethyl-7-oxabicyclo[13.2.1]octadeca-10,12-diene-6,14-dione (tylosin A aldol).

01/2008:1274

TYLOSIN TARTRATE FOR VETERINARY USE

Tylosini tartras ad usum veterinarium



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

DEFINITION

Tartrate of a 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-glucopyranosyl]oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-diene-2,10-dione (tylosin A, tartrate M_r 1982). Tylosin B (desmycosin, tartrate M_r 1694), tylosin C (macrocin, tartrate M_r 1954) and tylosin D (relomycin, tartrate M_r 1986) may also be present. They contribute to the potency of the substance to be examined.

Potency: minimum 800 IU/mg (dried substance).

CHARACTERS

Appearance: almost white or slightly yellow, hygroscopic powder.

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

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of tylosin tartrate.*

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. A green colour is produced.

TESTS

pH (*2.2.3*): 5.0 to 7.2.

Dissolve 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 it is intended for use in the manufacture of parenteral preparations.

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