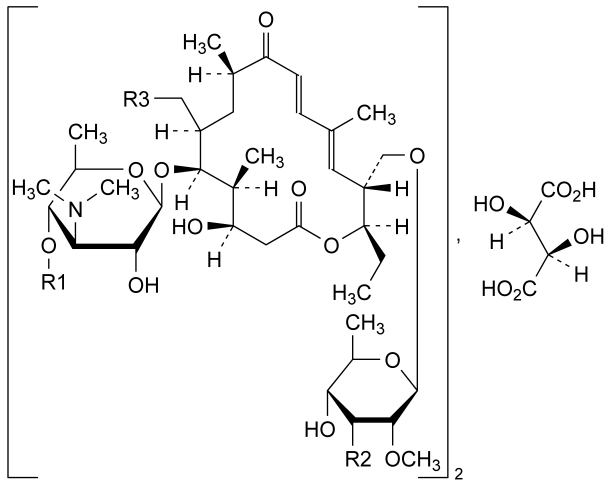
01/2008:1274

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 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 4.5 per cent, determined on 1.000 g by drying at 60 °C at a pressure not exceeding 0.7 kPa for 3 h.

Sulphated ash (*2.4.14*): maximum 2.5 per cent, determined on 1.0 g.

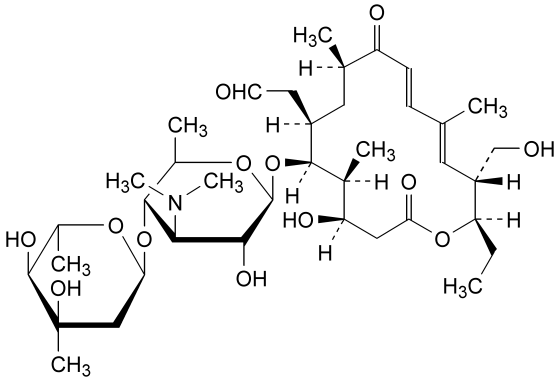
ASSAY

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

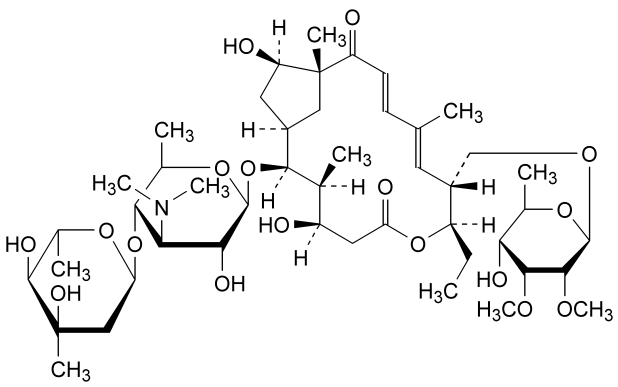
STORAGE

In an airtight container, protected from light.

IMPURITIES



A. desmycinosyltylosin,



B. tylosin A aldol.

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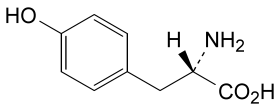
corrected 6.0

TYROSINE

Tyrosinum



$C_9H_{11}NO_3$ M_r 181.2
[60-18-4]

DEFINITION

Tyrosine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white crystalline powder or colourless crystals, very slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D, E.

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *tyrosine CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To about 50 mg add 1 ml of *dilute nitric acid R*. A dark red colour is produced within 15 min.

E. Dissolve about 30 mg in 2 ml of *dilute sodium hydroxide solution R*. Add 3 ml of a freshly prepared mixture of equal volumes of a 100 g/l solution of *sodium nitrite R* and a solution of 0.5 g of *sulphanilic acid R* in a mixture of 6 ml of *hydrochloric acid R1* and 94 ml of *water R*. An orange-red colour is produced.

TESTS

Appearance of solution. Dissolve 0.5 g in *dilute hydrochloric acid R* and dilute to 20 ml with the same acid. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dissolve 1.25 g in a mixture of equal volumes of *dilute hydrochloric acid R* and *water R* and dilute to 25.0 ml with the same mixture of solvents. The specific optical rotation is – 11.0 to – 12.3, calculated with reference to the dried substance.

Ninhydrin-positive substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *dilute ammonia R2* and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 50 ml with *water R*.