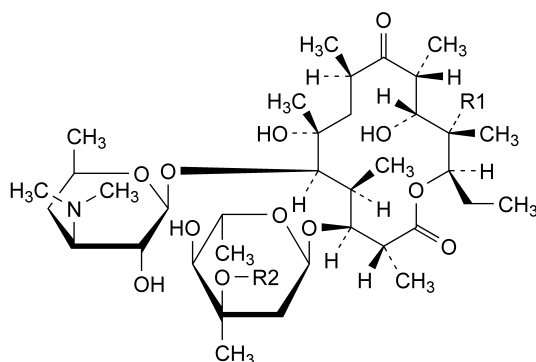


- C. (2*R*,3*S*,4*S*)-pentane-1,2,3,4,5-pentol (*meso*-ribose),
D. glycerol.

01/2008:0179
corrected 6.0

ERYTHROMYCIN

Erythromycinum



Erythromycin	Mol. Formula	M_r	R1	R2
A	$C_{37}H_{67}NO_{13}$	734	OH	CH_3
B	$C_{37}H_{67}NO_{12}$	718	H	CH_3
C	$C_{36}H_{65}NO_{13}$	720	OH	H

DEFINITION

Mixture of macrolide antibiotics produced by a strain of *Streptomyces erythreus*, the main component being (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[(3,4,6-trideoxy-3-dimethylamino- β -*D*-xylo-hexopyranosyl)oxy]oxacyclotetradecane-2,10-dione (erythromycin A).

Content:

- sum of the contents of erythromycin A, erythromycin B and erythromycin C: 93.0 per cent to 102.0 per cent (anhydrous substance),
- erythromycin B: maximum 5.0 per cent,
- erythromycin C: maximum 5.0 per cent.

CHARACTERS

Appearance: white or slightly yellow powder or colourless or slightly yellow crystals, slightly hygroscopic.

Solubility: slightly soluble in water (the solubility decreases as the temperature rises), freely soluble in alcohol, soluble in methanol.

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison: erythromycin A CRS.

Disregard any band in the region from 1980 cm^{-1} to 2050 cm^{-1} .

If the spectra obtained show differences, dissolve 50 mg of the substance to be examined and of the reference substance separately in 1.0 ml of *methylene chloride R*, dry at $60\text{ }^\circ\text{C}$ at a pressure not exceeding 670 Pa for 3 h and record new spectra using the residues.

B. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of *erythromycin A CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 20 mg of *spiramycin CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate: TLC silica gel G plate R.

Mobile phase: mix 4 volumes of *2-propanol R*, 8 volumes of a 150 g/l solution of *ammonium acetate R* previously adjusted to pH 9.6 with *ammonia R* and 9 volumes of *ethyl acetate R*. Allow to settle and use the upper layer.

Application: 10 μ l.

Development: over 2/3 of the plate.

Drying: in air.

Detection: spray with *anisaldehyde solution R1* and heat at $110\text{ }^\circ\text{C}$ for 5 min.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a) and its position and colour are different from those of the spots in the chromatogram obtained with reference solution (b).

C. To about 5 mg add 5 ml of a 0.2 g/l solution of *xanthidrol R* in a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *acetic acid R* and heat on a water-bath. A red colour develops.

D. Dissolve about 10 mg in 5 ml of *hydrochloric acid R1* and allow to stand for 10-20 min. A yellow colour develops.

TESTS

Specific optical rotation (2.2.7): -71 to -78 (anhydrous substance).

Dissolve 1.00 g in *ethanol R* and dilute to 50.0 ml with the same solvent. The specific optical rotation is determined at least 30 min after preparing the solution.

Related substances. Liquid chromatography (2.2.29).

Test solution. Dissolve 40.0 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (a). Dissolve 40.0 mg of *erythromycin A CRS* in a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1* and dilute to 10.0 ml with the same mixture of solvents.

Reference solution (b). Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1* and dilute to 50.0 ml with the same mixture of solvents.

Reference solution (c). Dissolve 5 mg of *N-demethyl-erythromycin A CRS* in reference solution (b). Add 1.0 ml of reference solution (a) and dilute to 25 ml with reference solution (b).

Reference solution (d). Dilute 3.0 ml of reference solution (a) to 100.0 ml with a mixture of 1 volume of *methanol R* and 3 volumes of *phosphate buffer solution pH 7.0 R1*.

Reference solution (e). Transfer 40 mg of erythromycin A CRS to a glass vial and spread evenly such that it forms a layer not more than about 1 mm thick. Heat at 130 °C for 4 h. Allow to cool and dissolve in a mixture of 1 volume of methanol R and 3 volumes of phosphate buffer solution pH 7.0 RI and dilute to 10 ml with the same mixture of solvents.

Column:

- size: $l = 0.25$ m, $\varnothing = 4.6$ mm,
- stationary phase: styrene-divinylbenzene copolymer R (8 μ m) with a pore size of 100 nm,
- temperature: 70 °C using a water-bath for the column and at least one-third of the tubing preceding the column.

Mobile phase: to 50 ml of a 35 g/l solution of dipotassium hydrogen phosphate R adjusted to pH 9.0 \pm 0.05 with dilute phosphoric acid R, add 400 ml of water R, 165 ml of 2-methyl-2-propanol R and 30 ml of acetonitrile R, and dilute to 1000 ml with water R.

Flow rate: 2.0 ml/min.

Detection: spectrophotometer at 215 nm.

Injection: 100 μ l; inject the test solution and reference solutions (c), (d) and (e).

Run time: 5 times the retention time of erythromycin A.

Relative retention with reference to erythromycin A (retention time = about 15 min): impurity A = about 0.3; impurity B = about 0.45; erythromycin C = about 0.5; impurity C = about 0.9; impurity D = about 1.4; impurity F = about 1.5; erythromycin B = about 1.8; impurity E = about 4.3.

System suitability: reference solution (c):

- resolution: minimum 0.8 between the peaks due to impurity B and erythromycin C and minimum 5.5 between the peaks due to impurity B and erythromycin A. If necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase or reduce the flow rate to 1.5 ml or 1.0 ml/min.

Limits:

- correction factors: for the calculation of contents, multiply the peak areas of the following impurities (use the chromatogram obtained with reference solution (e) to identify them) by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15,
- any impurity: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent),
- total: not more than 2.3 times the area of the principal peak in the chromatogram obtained with reference solution (d) (7.0 per cent),
- disregard limit: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.06 per cent); disregard the peaks due to erythromycin B and erythromycin C.

Thiocyanate: maximum 0.3 per cent.

Prepare the solutions immediately before use and protect from actinic light.

Compensation liquid. Dilute 1.0 ml of a 90 g/l solution of ferric chloride R to 50.0 ml with methanol R.

Test solution. Dissolve 0.100 g (m g) of the substance to be examined in 20 ml of methanol R, add 1.0 ml of a 90 g/l solution of ferric chloride R and dilute to 50.0 ml with methanol R.

Prepare 2 independent reference solutions.

Reference solution. Dissolve 0.100 g of potassium thiocyanate R, previously dried at 105 °C for 1 h, in methanol R and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml to 50.0 ml with methanol R. To 5.0 ml of this solution, add 1.0 ml of a 90 g/l solution of ferric chloride R and dilute to 50.0 ml with methanol R.

Measure the absorbances (2.2.25) of each reference solution (A_1 , A_2) and of the test solution (A) at the maximum (about 492 nm).

Suitability value:

$$S = \frac{m_2 \times A_1}{m_1 \times A_2}$$

m_1 , m_2 = mass of the potassium thiocyanate used to prepare the respective reference solutions, in grams.

The test is not valid unless S is not less than 0.985 and not more than 1.015.

Calculate the percentage content of thiocyanate from the expression:

$$\frac{A \times 58.08 \times 0.5}{m \times 97.18} \times \left(\frac{m_1}{A_1} + \frac{m_2}{A_2} \right)$$

58.08 = relative molecular mass of the thiocyanate moiety,

97.18 = relative molecular mass of potassium thiocyanate.

Water (2.5.12): maximum 6.5 per cent, determined on 0.200 g.

Use a 100 g/l solution of imidazole R in anhydrous methanol R as the solvent.

Sulphated ash (2.4.14): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (2.2.29) as described in the test for related substances with the following modifications.

Injection: test solution and reference solutions (a) and (b).

System suitability: reference solution (a):

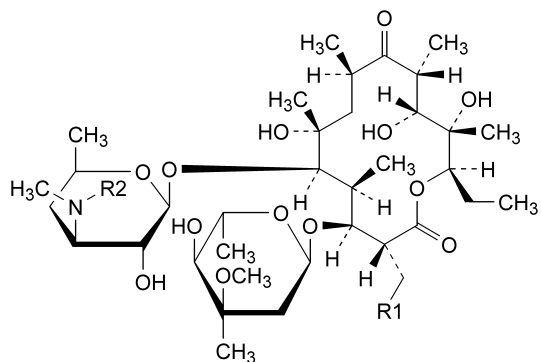
- repeatability: maximum relative standard deviation of 1.2 per cent for 6 replicate injections.

Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b).

STORAGE

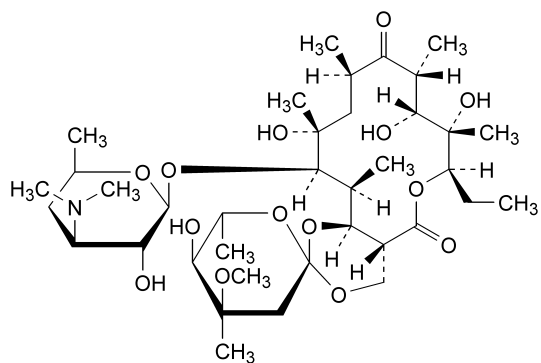
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IMPURITIES

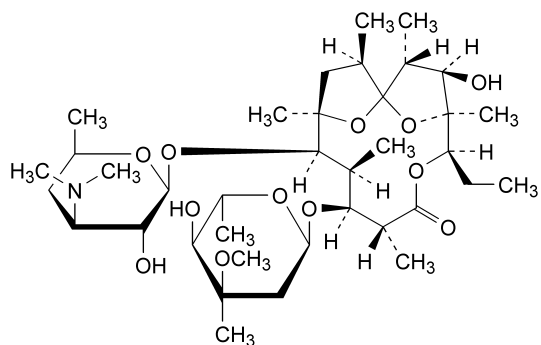


A. R1 = OH, R2 = CH₃: erythromycin F,

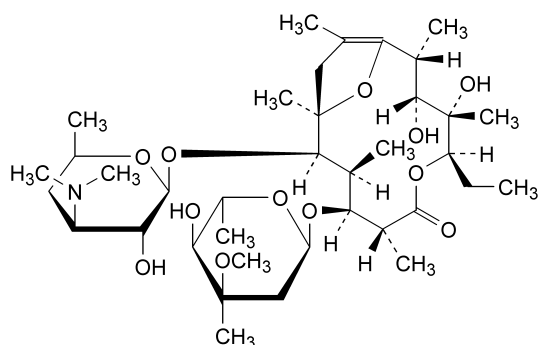
B. R1 = R2 = H: *N*-demethylethromycin A,



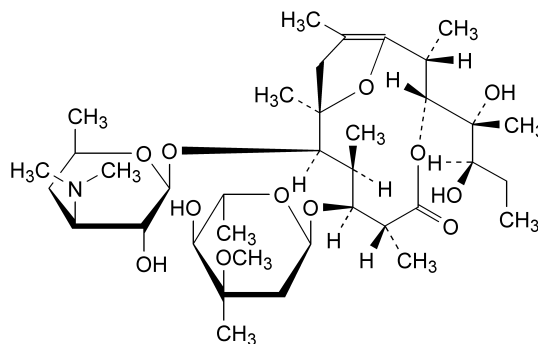
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,

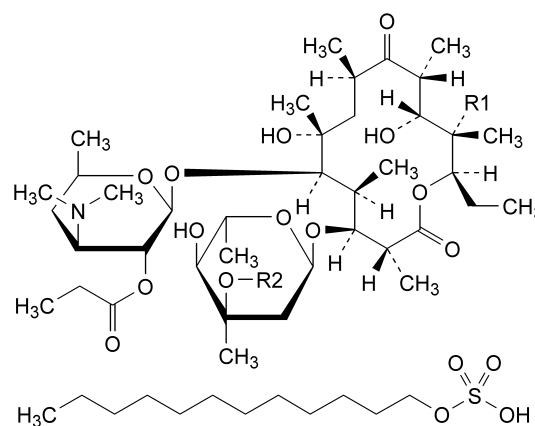


F. pseudoerythromycin A enol ether.

01/2008:0552

ERYTHROMYCIN ESTOLATE

Erythromycini estolas



Erythromycin (estolate)	Mol. Formula	M _r	R1	R2
A	C ₅₂ H ₉₇ NO ₁₈ S	1056	OH	CH ₃
B	C ₅₂ H ₉₇ NO ₁₇ S	1040	H	CH ₃
C	C ₅₁ H ₉₅ NO ₁₈ S	1042	OH	H

DEFINITION

Main component: (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribohexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-2-*O*-propionyl- β -D-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione dodecyl sulphate (erythromycin A 2''-propionate dodecyl sulphate).

Semi-synthetic product derived from a fermentation product.

Content:

- *erythromycin estolate:* 86.0 per cent to 102.0 per cent (anhydrous substance),
- *erythromycin B:* maximum 5.0 per cent (anhydrous substance),
- *erythromycin C:* maximum 5.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.
Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in acetone. It is practically insoluble in dilute hydrochloric acid.

IDENTIFICATION

Infrared absorption spectrophotometry (2.2.24).