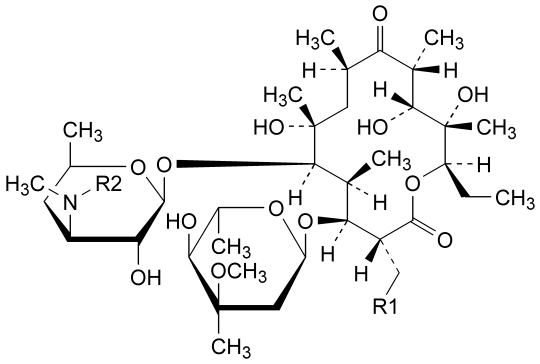
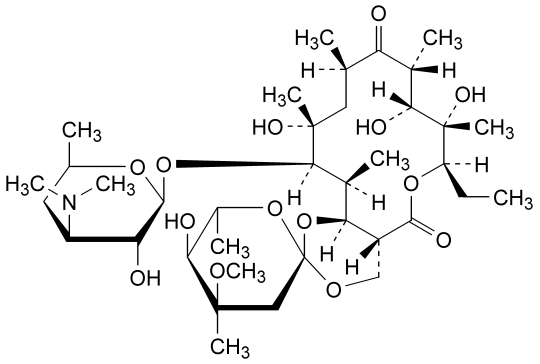
IMPURITIES

Specified impurities: A, B, C, D, E, F.

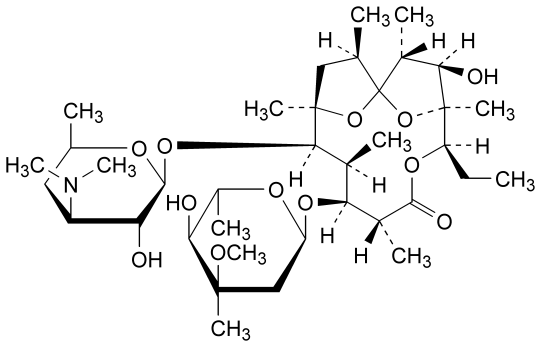


A. R1 = OH, R2 = CH_3: erythromycin F,

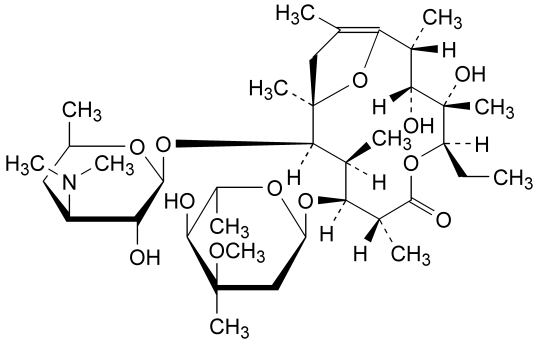
B. R1 = R2 = H: *N*-demethylerythromycin A,



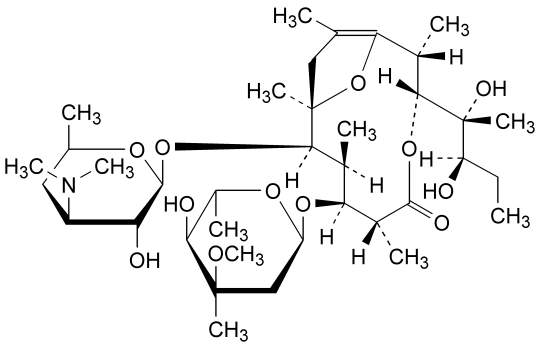
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,

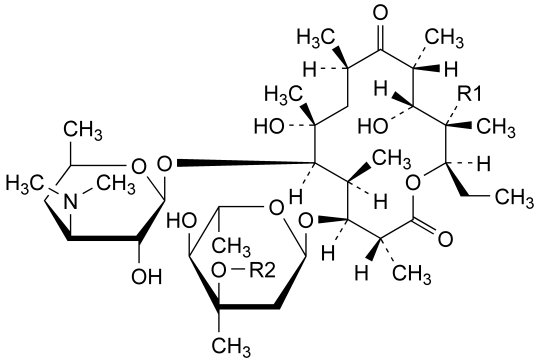


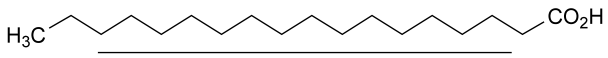
F. pseudoerythromycin A enol ether.

01/2008:0490
corrected 6.0

ERYTHROMYCIN STEARATE

Erythromycini stearas





Erythromycin	Mol. Formula	R1	R2
A	$C_{55}H_{103}NO_{15}$	OH	CH_3
B	$C_{55}H_{103}NO_{14}$	H	CH_3
C	$C_{54}H_{101}NO_{15}$	OH	H

$C_{55}H_{103}NO_{15}$ M_r 1018

DEFINITION

A mixture of the stearates of erythromycin and stearic acid. The main component is the octadecanoate of (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 stearate).

Fermentation product.

Content:

– sum of the contents of erythromycin A, erythromycin B and erythromycin C: minimum 60.5 per cent (anhydrous substance),
– erythromycin B: maximum 5.0 per cent,
– erythromycin C: maximum 5.0 per cent.

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, soluble in acetone and in methanol.

Solutions may be opalescent.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *erythromycin stearate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 28 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

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

Reference solution (b). Dissolve 10 mg of *stearic acid R* 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: 5 µl.

Development: over 2/3 of the plate.

Drying: in air.

Detection A: spray with a solution containing 0.2 g/l of *dichlorofluorescein R* and 0.1 g/l of *rhodamine B R* in *alcohol R*. Maintain the plate for a few seconds in the vapour above a water-bath. Examine in ultraviolet light at 365 nm.

Results A: the chromatogram obtained with the test solution shows 2 spots, one of which corresponds in position to the principal spot in the chromatogram obtained with reference solution (a) and the other to the principal spot in the chromatogram obtained with reference solution (b).

Detection B: spray the plate with *anisaldehyde solution R1*. Heat at 110 °C for 5 min and examine in daylight.

Results B: the spot in the chromatogram obtained with the test solution corresponds in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Free stearic acid: maximum 14.0 per cent (anhydrous substance) of $C_{18}H_{36}O_2$.

Dissolve 0.400 g in 50 ml of *methanol R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*). Calculate the volume of *0.1 M sodium hydroxide* required per gram of the substance to be examined (n_1 ml). Dissolve 0.500 g in 30 ml of *methylene chloride R*. If the solution is opalescent, filter and shake the residue with 3 quantities, each of 25 ml, of *methylene chloride R*. Filter, if necessary, and rinse the filter with *methylene chloride R*. Reduce the volume of the combined filtrate and rinsings to 30 ml by evaporation on a water-bath. Add 50 ml of *glacial acetic acid R* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Calculate the volume of *0.1 M perchloric acid* required per gram of the substance to be examined (n_2 ml).

Calculate the percentage content of $C_{18}H_{36}O_2$ from the expression:

$$2.845\,(n_1 - n_2) \times \frac{100}{100 - h}$$

h = percentage water content.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 55.0 mg of the substance to be examined in 5.0 ml of *methanol R* and dilute to 10.0 ml with *buffer solution pH 8.0 R1*. Centrifuge and use the clear solution.

Reference solution (a). Dissolve 40.0 mg of *erythromycin A CRS* in 5.0 ml of *methanol R* and dilute to 10.0 ml with *buffer solution pH 8.0 R1*.

Reference solution (b). Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in 25.0 ml of *methanol R* and dilute to 50.0 ml with *buffer solution pH 8.0 R1*.

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 equal volumes of *methanol R* and *buffer solution pH 8.0 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 *buffer solution pH 8.0 R1* and dilute to 10 ml with the same mixture of solvents.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm) 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 ± 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 µl; 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 to erythromycin C and minimum 5.5 between the peaks due to impurity B and to 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/min 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 per cent),

– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (6 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 to erythromycin C.

Water (*2.5.12*): maximum 4.0 per cent, determined on 0.300 g.

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

Sulphated ash (*2.4.14*): maximum 0.5 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.

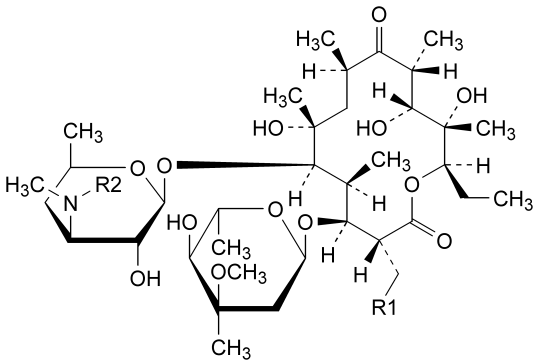
Injection: test solution and reference solutions (a) and (b).

System suitability: reference solution (a):

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

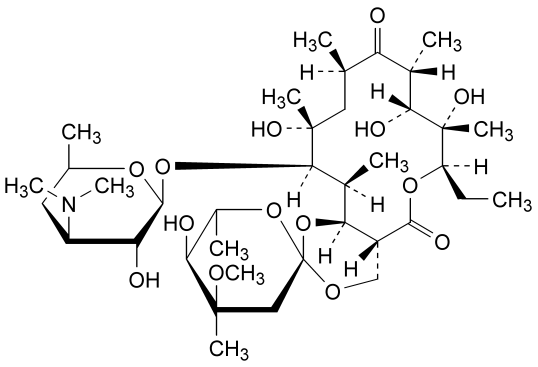
Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b).

IMPURITIES

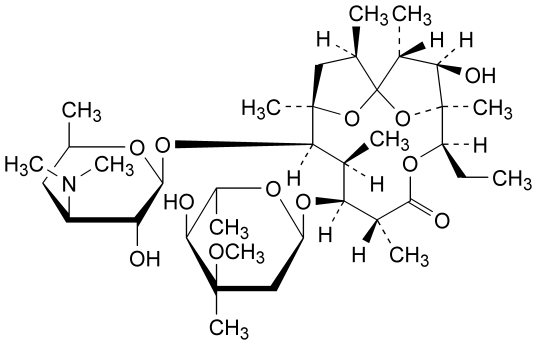


A. R1 = OH, R2 = CH_3: erythromycin F,

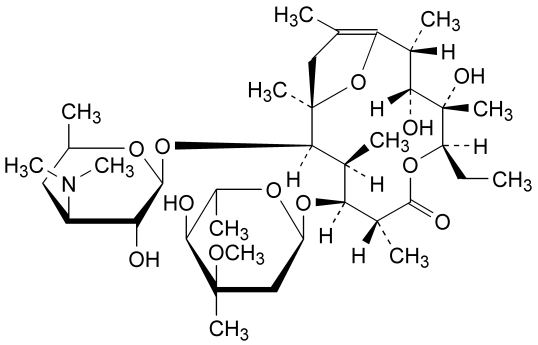
B. R1 = R2 = H: *N*-demethylerythromycin A,



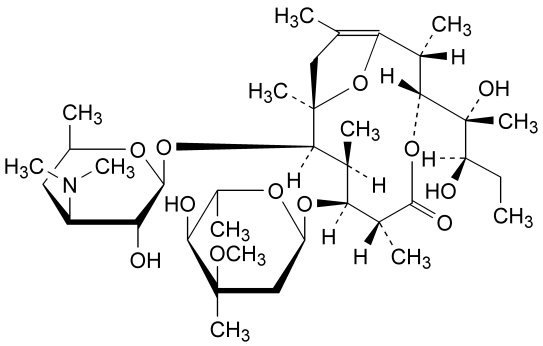
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,



F. pseudoerythromycin A enol ether.