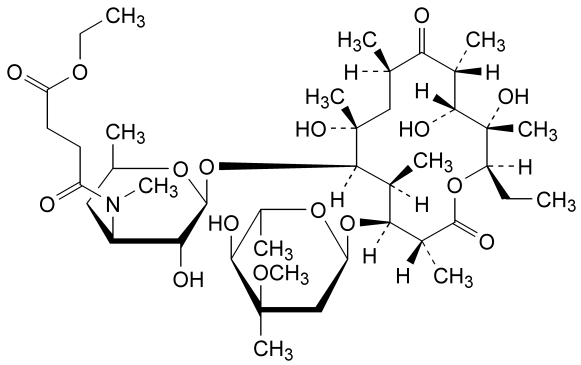
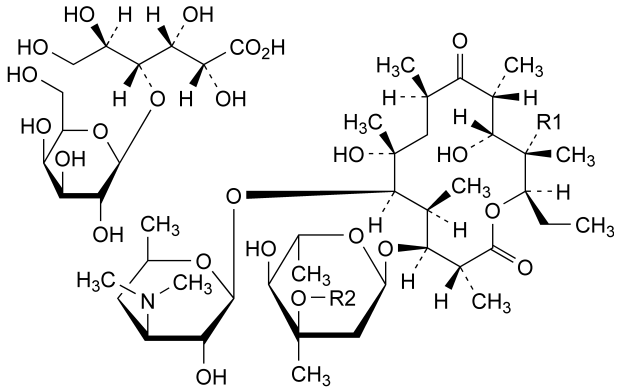


G. erythromycin *N*-ethylsuccinate.

01/2008:1098

ERYTHROMYCIN LACTOBIONATE

Erythromycini lactobionas



Erythromycin (lactobionate)	Mol. Formula	M_r	R1	R2
A	$C_{49}H_{89}NO_{25}$	1092	OH	CH_3
B	$C_{49}H_{89}NO_{24}$	1076	H	CH_3
C	$C_{48}H_{87}NO_{25}$	1078	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-*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 4-*O*-β-D-galactopyranosyl-D-gluconate (erythromycin A lactobionate).

Salt of a product obtained by fermentation using a strain of *Streptomyces erythreus*.

Content:

– *sum of erythromycin A lactobionate, erythromycin B lactobionate and erythromycin C lactobionate*: 93.0 per cent to 102.0 per cent (anhydrous substance);

– *erythromycin B lactobionate*: maximum 5.0 per cent (anhydrous substance);

– *erythromycin C lactobionate*: maximum 5.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or slightly yellow hygroscopic, powder.

Solubility: soluble in water, freely soluble in anhydrous ethanol and in methanol, very slightly soluble in acetone and in methylene chloride.

IDENTIFICATION

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

Test solution. Dissolve 30 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 *lactobionic acid R* in *water R* and dilute to 10 ml with the same solvent.

Plate: *TLC silica gel plate R.*

Mobile phase: *glacial acetic acid R, water R, methanol R* (3:10:90 *V/V/V*).

Application: 5 µl.

Development: over 3/4 of the plate.

Drying: in air.

Detection: spray with a 5 g/l solution of *potassium permanganate R* in *1 M sodium hydroxide* and heat at 110 °C for 5 min.

Results: the 2 spots in the chromatogram obtained with the test solution are similar in position, colour and size, one 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).

B. To about 5 mg add 5 ml of a 0.2 g/l solution of *xanthydrol R* in a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *acetic acid R*. A red colour develops.

C. Dissolve about 10 mg in 5 ml of *hydrochloric acid R1*. A yellowish-green colour develops.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 1.0 g in 20 ml of *water R*.

pH (*2.2.3*): 6.5 to 7.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *The test solution and the reference solutions can be used within 24 h if stored at 2-8 °C.*

Solvent mixture: *methanol R, phosphate buffer solution pH 7.0 R* (25:75 *V/V*).

Test solution. Dissolve 60.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). Dissolve 40.0 mg of *erythromycin A CRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (b). Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *N-demethylerythromycin A CRS* (impurity B) 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 the solvent mixture.

Reference solution (e). Dissolve 40 mg of *erythromycin A CRS*, previously heated at 130 °C for 4 h, in the solvent mixture and dilute to 10 ml with the solvent mixture (*in situ* preparation of impurities E and F).

Reference solution (f). Dissolve 2 mg of *erythromycin A CRS* in 5 ml of *0.01 M hydrochloric acid*. Allow to stand at room temperature for 30 min. Dilute to 10 ml with the solvent mixture (*in situ* preparation of impurity D).

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 1/3 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 with *dilute phosphoric acid R*, add 400 ml of *water R*, 165 ml of *2-methyl-2-propanol R* and 30 ml of *acetonitrile R1*, and dilute to 1000 ml with *water R*.

Flow rate: 2.0 ml/min.

Detection: spectrophotometer at 215 nm.

Injection: 100 µl of the test solution and reference solutions (a), (c), (d), (e) and (f).

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

Identification of impurities: use the chromatogram obtained with reference solution (c) to identify the peak due to impurity B, with reference solution (e) to identify the peaks due to impurities E and F, and with reference solution (f) to identify the peak due to impurity D.

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/min or 1.0 ml/min.

Limits:

– *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15;

– *impurities A, B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent);

– *any other impurity*: for each impurity, not more than 0.067 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent);

– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (6.0 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).

Free lactobionic acid: maximum 1.0 per cent of $C_{12}H_{22}O_{12}$ (anhydrous substance).

Dissolve 0.400 g in 50 ml of *water 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 40 ml of *anhydrous 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 $C_{12}H_{22}O_{12}$ using the following expression:

$$3.580\,(n_1 - n_2)$$

Water (*2.5.12*): maximum 5.0 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 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*): less than 0.35 IU/mg of erythromycin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

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:

– *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (a).

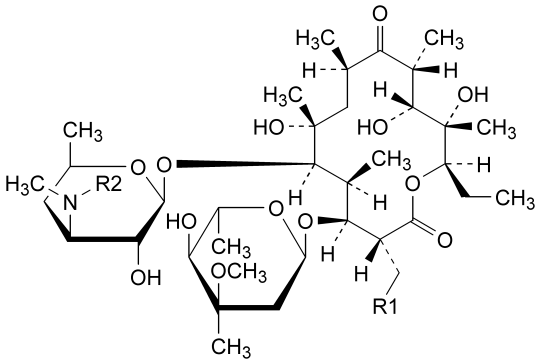
Calculate the percentage content of erythromycin A using the chromatogram obtained with reference solution (a). Express the result as erythromycin A lactobionate by multiplying the percentage content of erythromycin A by 1.4877. Calculate the percentage contents of erythromycin B and erythromycin C using the chromatogram obtained with reference solution (b). Express the result as erythromycin B lactobionate and as erythromycin C lactobionate by multiplying by 1.4877.

STORAGE

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

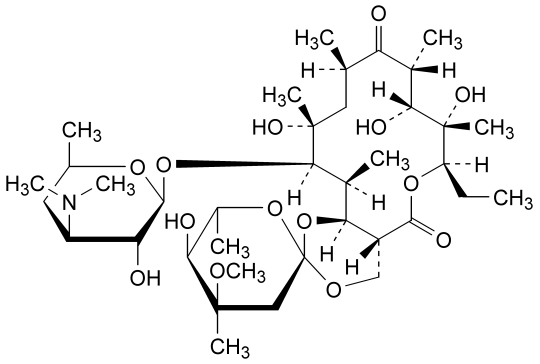
IMPURITIES

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

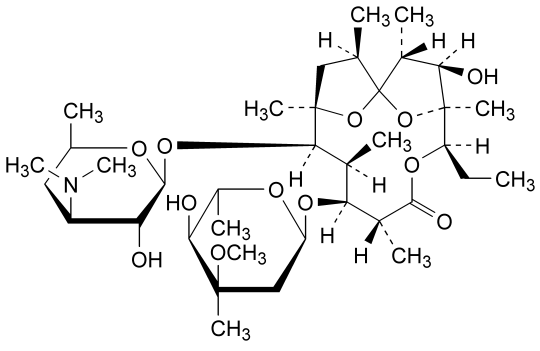


A. R1 = OH, R2 = CH_3: erythromycin F,

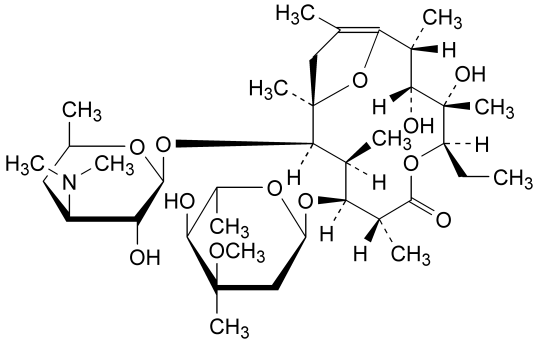
B. R1 = R2 = H: *N*-demethylerythromycin A,



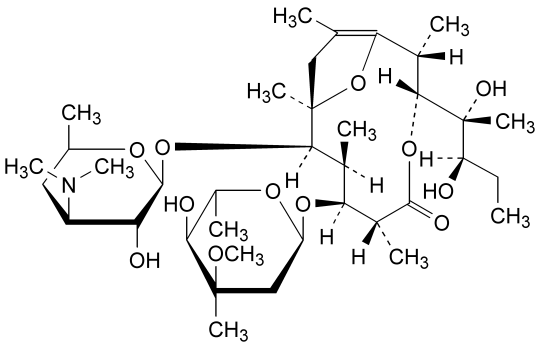
C. erythromycin E,



D. anhydroerythromycin A,



E. erythromycin A enol ether,

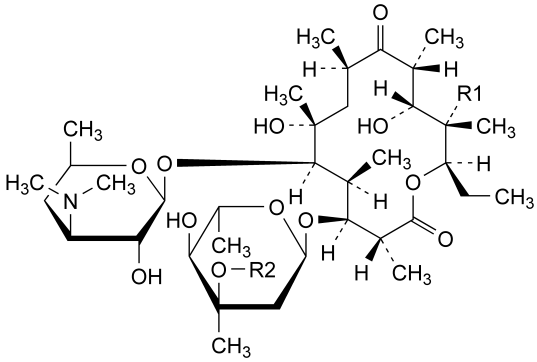


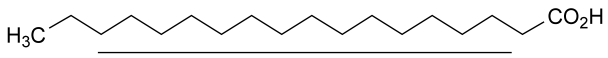
F. pseudoerythromycin A enol ether.

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.