Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a). Dissolve 40 mg of the substance to be examined in *acetone R* and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 2.5 ml of test solution (a) to 10 ml with *acetone R*.

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

Reference solution (b). Dissolve 10 mg of *erythromycin estolate CRS* and 10 mg of *erythromycin ethylsuccinate CRS* in *acetone R* and dilute to 10 ml with the same solvent.

Reference solution (c). Dissolve 8 mg of *erythromycin CRS* in *acetone R* and dilute to 100 ml with the same solvent.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 1 volume of a 150 g/l solution of *ammonium acetate R* previously adjusted to pH 7.0, 15 volumes of *alcohol R* and 85 volumes of *chloroform R*. Allow the plate to dry in air and spray with *anisaldehyde solution R*. Heat at 110 °C for 5 min and allow to cool. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (c) (2.0 per cent).

Dodecyl sulphate. 23.0 per cent to 25.5 per cent of $C_{12}H_{26}O_4S$, calculated with reference to the anhydrous substance. Dissolve 0.500 g of the substance to be examined in 25 ml of *dimethylformamide R*. Titrate with *0.1 M sodium methoxide* using 0.05 ml of a 3 g/l solution of *thymol blue R* in *methanol R* as indicator.

1 ml of *0.1 M sodium methoxide* is equivalent to 26.64 mg of $C_{12}H_{26}O_4S$.

Water (*2.5.12*). Not more than 4.0 per cent, determined on 0.300 g by the semi-micro determination of water. Use a 100 g/l solution of *imidazole R* in *anhydrous methanol R* as the solvent.

Sulphated ash (*2.4.14*). Not more than 0.5 per cent, determined on 0.5 g.

ASSAY

Dissolve 40.0 mg in 40 ml of *methanol R*, add 20 ml of *phosphate buffer solution pH 7.0 R* and dilute to 100.0 ml with *water R*. Maintain at 60 °C for 3 h and allow to cool. Carry out the microbiological assay of antibiotics (*2.7.2*). Use *erythromycin CRS* as the reference substance.

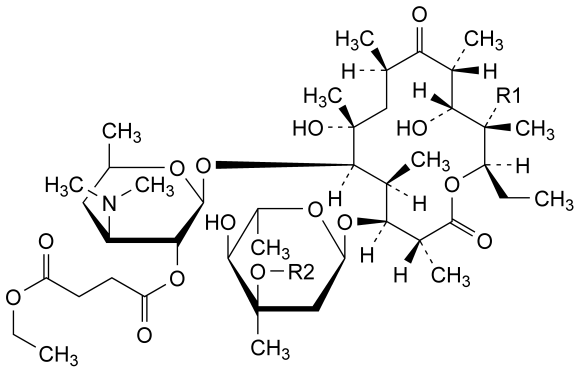
STORAGE

Store in an airtight container, protected from light, at a temperature below 30 °C.

01/2005:0274

ERYTHROMYCIN ETHYLSUCCINATE

Erythromycini ethylsuccinas



Ethylsuccinate compound	Mol. Formula	M_r	R1	R2
Erythromycin A	$C_{43}H_{75}NO_{16}$	862	OH	CH_3
Erythromycin B	$C_{43}H_{75}NO_{15}$	846	H	CH_3
Erythromycin C	$C_{42}H_{73}NO_{16}$	848	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)-2-*O*-(4-ethoxy-4-oxobutanoyl)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (erythromycin A ethylsuccinate).

Content:

– sum of erythromycin A, erythromycin B and erythromycin C: minimum 78.0 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, crystalline powder, hygroscopic.

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

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *erythromycin ethylsuccinate CRS*.

TESTS

Specific optical rotation (*2.2.7*): −70 to −82 (anhydrous substance).

Dissolve 0.100 g in *acetone R* and dilute to 10.0 ml with the same solvent. Measure the angle of rotation at least 30 min after preparing the solution.

Related substances. Liquid chromatography (*2.2.29*).

Hydrolysis solution. A 20 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *phosphoric acid R*.

Test solution. Dissolve 0.115 g of the substance to be examined in 25 ml of *methanol R*. Add 20 ml of the hydrolysis solution, mix and allow to stand at room temperature for at least 12 h. Dilute to 50.0 ml with the hydrolysis solution.

Reference solution (a). Dissolve 40.0 mg of *erythromycin A CRS* in 10 ml of *methanol R* and dilute to 20.0 ml with the hydrolysis solution.

Reference solution (b). Dissolve 10.0 mg of *erythromycin B CRS* and 10.0 mg of *erythromycin C CRS* in 50 ml of *methanol R*. Add 5.0 ml of reference solution (a) and dilute to 100.0 ml with the hydrolysis solution.

Reference solution (c). Dissolve 2 mg of *N-demethylerythromycin A CRS* in 20 ml of 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 the hydrolysis solution.

Reference solution (e). Dissolve 40 mg of *erythromycin A CRS*, previously heated at 130 °C for 3 h, in 10 ml of *methanol R* and dilute to 20 ml with the hydrolysis solution.

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

Flow rate: 2.0 ml/min.

Detection: spectrophotometer at 215 nm.

Injection: 200 µl; inject the test solution and reference solutions (a), (c), (d) and (e).

Run time: 5 times the retention time of erythromycin A; begin integration after the hydrolysis peak.

Relative retention with reference to erythromycin A (retention time = about 15 min): hydrolysis peak = less than 0.3; impurity B = about 0.45; erythromycin C = about 0.5; impurity C = about 0.9; impurity G = about 1.3; 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.

Limits:

– *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity E = 0.09; impurity F = 0.15; impurity G = 0.14; use the chromatogram obtained with reference solution (e) to identify the peaks due to impurities E and F.

– *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 1.67 times the area of the principal peak in the chromatogram obtained with reference solution (d) (5.0 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 erythromycin. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.250 g of the substance to be examined in *acetonitrile R* and dilute to 50.0 ml with the same solvent.

Reference solution. Dissolve 75.0 mg of *erythromycin A CRS* in *acetonitrile R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *acetonitrile R*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,

– *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 35 volumes of *acetonitrile R* and 65 volumes of a solution containing 3.4 g/l of *potassium dihydrogen phosphate R* and 2.0 g/l of *triethylamine R*, adjusted to pH 3.0 with *dilute phosphoric acid R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 195 nm.

Injection: 20 µl.

Run time: twice the retention time of erythromycin A (retention time = about 8 min) for the reference solution and twice the retention time of erythromycin ethylsuccinate (retention time = about 24 min) for the test solution.

Limit:

– *free erythromycin*: not more than the area of the principal peak in the chromatogram obtained with the reference solution (6.0 per cent).

Water (*2.5.12*): maximum 3.0 per cent, determined on 0.30 g.

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

Sulphated ash (*2.4.14*): maximum 0.3 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances.

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

System suitability: reference solution (a):

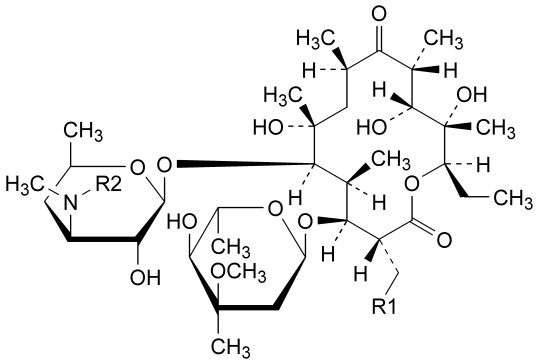
– *relative standard deviation*: maximum 1.2 per cent for 6 replicate injections.

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

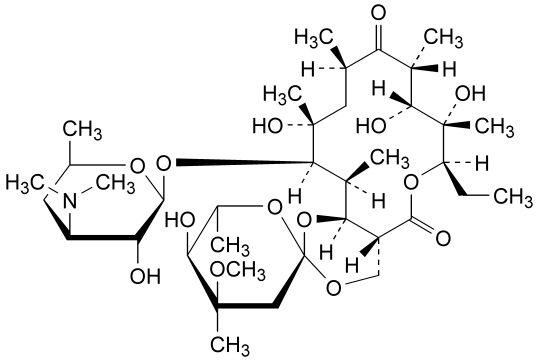
In an airtight container, protected from light.

IMPURITIES

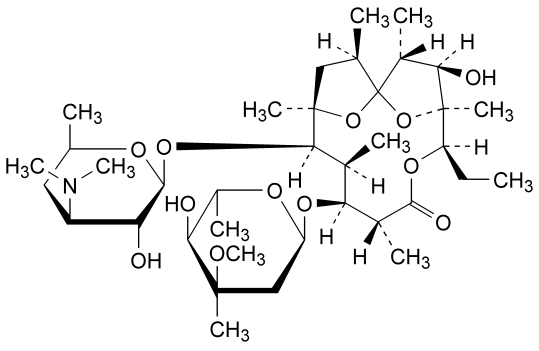


A. R1 = OH, R2 = CH_3: erythromycin F,

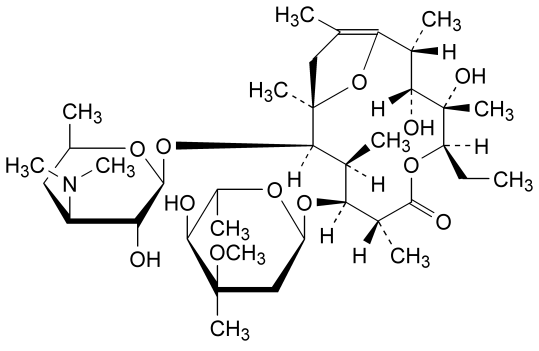
B. R1 = R2 = H: *N*-demethylerythromycin A,



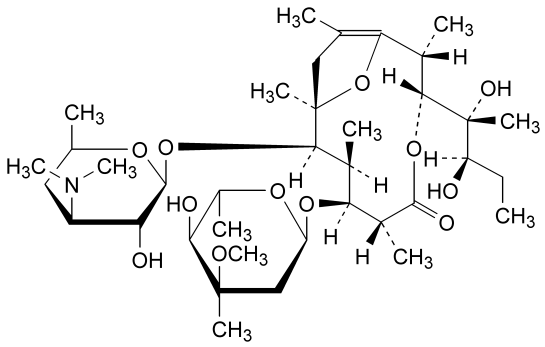
C. erythromycin E,



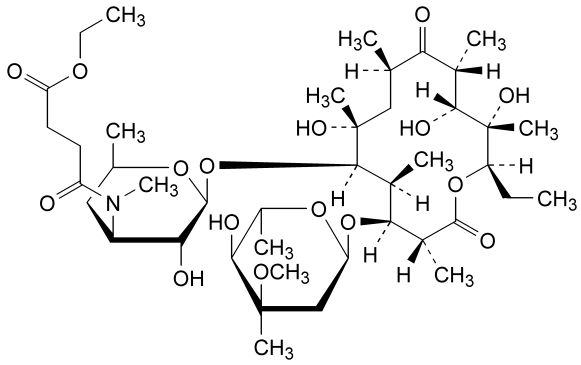
D. anhydroerythomycin A,



E. erythromycin A enol ether,



F. pseudoerythromycin A enol ether,

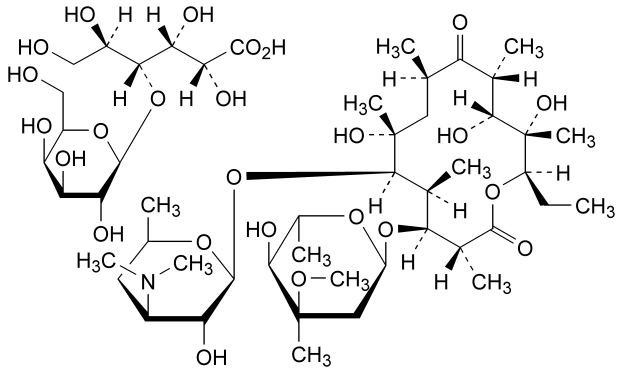


G. erythromycin *N*-ethylsuccinate.

01/2005:1098

ERYTHROMYCIN LACTOBIONATE

Erythromycini lactobionas



$C_{49}H_{89}NO_{25}$ M_r 1092

DEFINITION

Erythromycin lactobionate is a mixture of lactobionates of macrolide antibiotics. The main component is (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. The sum of the content of erythromycin A lactobionate, of erythromycin B lactobionate and of erythromycin C lactobionate is not less than 93.0 per cent and not more than the equivalent of 100.5 per cent, calculated with reference to the anhydrous substance.