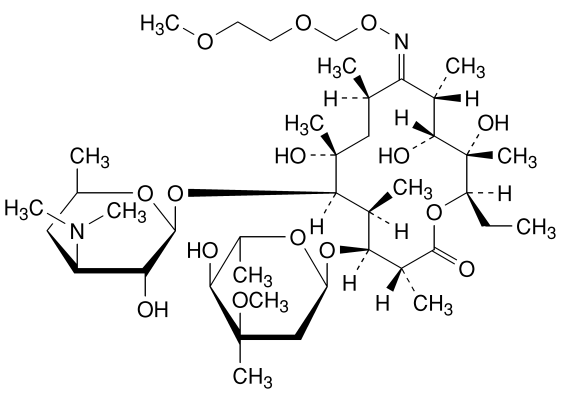
01/2008:1146
corrected 6.0

ROXITHROMYCIN

Roxithromycinum



$C_{41}H_{76}N_2O_{15}$ M_r 837
[80214-83-1]

DEFINITION

(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,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-10-[(*E*)-[(2-methoxyethoxy)methoxy]imino]-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecan-2-one (erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime]).

Semi-synthetic product derived from a fermentation product.

Content: 96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: very slightly soluble in water, freely soluble in acetone, in alcohol and in methylene chloride. It is slightly soluble in dilute hydrochloric acid.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *roxithromycin CRS*.

If the spectra obtained shows differences, prepare further spectra using 90 g/l solutions in *methylene chloride R*.

B. Examine the chromatograms obtained in the assay.

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).

TESTS

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

Dissolve 0.2 g in *methanol R* and dilute to 20 ml with the same solvent.

Specific optical rotation (*2.2.7*): – 93 to – 96 (anhydrous substance).

Dissolve 0.500 g in *acetone R* and dilute to 50.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Solution A. Mix 30 volumes of *acetonitrile R* and 70 volumes of a 48.6 g/l solution of *ammonium dihydrogen phosphate R*, adjusted to pH 5.3 with *dilute sodium hydroxide solution R*.

Test solution. Dissolve 50.0 mg of the substance to be examined in solution A and dilute to 25.0 ml with solution A.

Reference solution (a). Dissolve 50.0 mg of *roxithromycin CRS* in solution A and dilute to 25.0 ml with solution A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with solution A.

Reference solution (c). Dissolve 2.0 mg of *roxithromycin for system suitability CRS* in solution A and dilute to 1.0 ml with solution A.

Reference solution (d). Dilute 1.0 ml of *toluene R* to 100.0 ml with *acetonitrile R*. Dilute 0.2 ml of this solution to 200.0 ml with solution A.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,
– *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 μm) with a 10 nm pore size and a carbon loading of about 19 per cent,
– *temperature*: 15 °C.

Mobile phase:

– *mobile phase A*: mix 26 volumes of *acetonitrile R* and 74 volumes of a 59.7 g/l solution of *ammonium dihydrogen phosphate R*, adjusted to pH 4.3 with *dilute sodium hydroxide solution R*,
– *mobile phase B*: *water R, acetonitrile R* (30:70 *V/V*),

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 50	100	0
50 - 51	100 → 90	0 → 10
51 - 80	90	10
80 - 81	90 → 100	10 → 0
81 - 100	100	0

Flow rate: 1.1 ml/min.

Detection: spectrophotometer at 205 nm.

Injection: 20 μl, using an injector maintained at 8 °C, of the test solution and reference solutions (b), (c) and (d).

Relative retention with reference to roxithromycin (retention time = about 22 min): impurity A = about 0.28; impurity B = about 0.31; impurity C = about 0.33; impurity D = about 0.62; impurity E = about 0.67; impurity F = about 0.83; impurity G = about 1.15; impurity K = about 1.7; impurity H = about 1.85; impurity J = about 2.65; impurity I = about 3.1.

System suitability: reference solution (c):

– *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

Limits:

– *impurity G*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),

– *impurities A, B, C, D, E, F, H, I, J*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

– *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),

– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent). Disregard any peak due to toluene (use reference solution (d) to identify it).

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *acetone R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with the limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 15 volumes of *water R* and 85 volumes of *acetone R*.

Water (*2.5.12*): maximum 3.0 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*): maximum 0.1 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.

Column:

– *size*: *l* = 0.25 m.

Mobile phase: mix 307 volumes of *acetonitrile R* and 693 volumes of a 49.1 g/l solution of *ammonium dihydrogen phosphate R* adjusted to pH 5.3 with *dilute sodium hydroxide solution R*.

Flow rate: 1.5 ml/min.

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

Retention time: roxithromycin = about 12 min.

System suitability: reference solution (c):

– *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity G and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

STORAGE

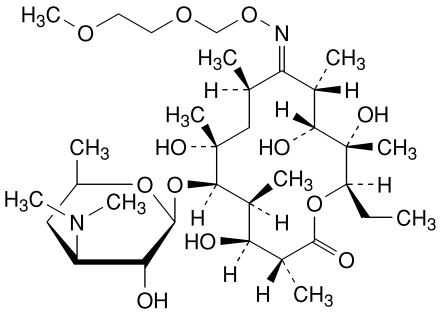
In an airtight container.

IMPURITIES

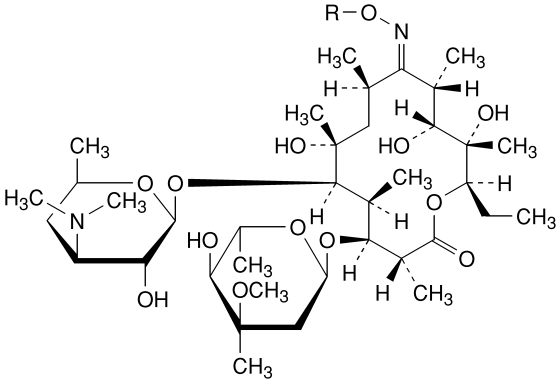
Specified impurities: A, B, C, D, E, F, G, H, I, J.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): K.

A. (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),



B. 3-*O*-de(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],

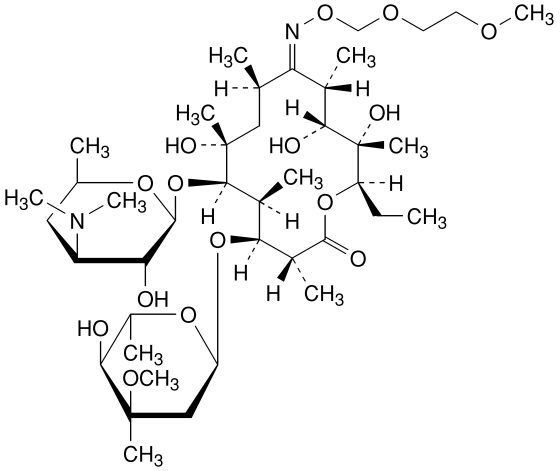


C. R = H: erythromycin 9-(*E*)-oxime,

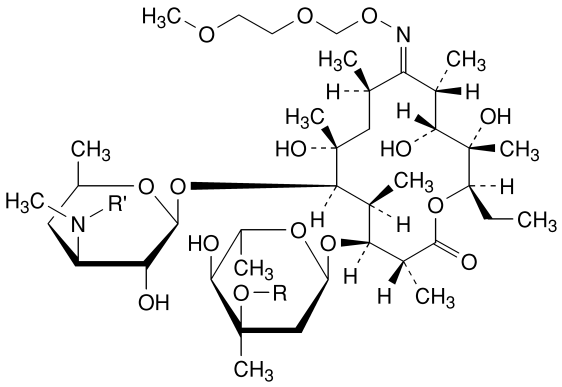
G. R = CH_2-O-CH_2-O-CH_2-CH_2-OCH_3: erythromycin 9-(*E*)-[*O*-[[(2-methoxyethoxy)methoxy]methyl]oxime],

J. R = CH_2-O-CH_2-CH_2Cl: erythromycin 9-(*E*)-[*O*-[(2-chloroethoxy)methyl]oxime],

K. R = CH_2-O-CH_2-CH_2-O-CH_2OH: erythromycin 9-(*E*)-[*O*-[[2-(hydroxymethoxy)ethoxy]methyl]oxime],

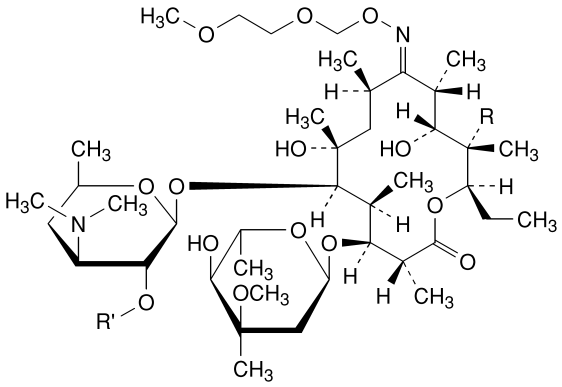


D. erythromycin 9-(*Z*)-[*O*-[(2-methoxyethoxy)methyl]oxime],



E. R = H, R′ = CH_3: 3″-*O*-demethylerythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],

F. R = CH_3, R′ = H: 3′-*N*-demethylerythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],



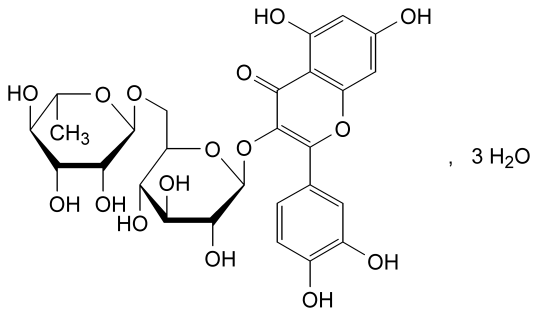
H. R = R′ = H: 12-deoxyerythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime],

I. R = OH, R′ = CH_2-O-CH_2-CH_2-OCH_3: 2′-*O*-[(2-methoxyethoxy)methyl]erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime].

01/2008:1795

RUTOSIDE TRIHYDRATE

Rutosidum trihydricum



$C_{27}H_{30}O_{16},3H_2O$ M_r 665

DEFINITION

3-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one.

Content: 95.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: yellow or greenish-yellow, crystalline powder.

Solubility: practically insoluble in water, soluble in methanol, sparingly soluble in ethanol, practically insoluble in methylene chloride. It dissolves in solutions of alkali hydroxides.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Dissolve 50.0 mg in *methanol R*, dilute to 250.0 ml with the same solvent and filter if necessary. Dilute 5.0 ml of the solution to 50.0 ml with *methanol R*. Examined between 210 nm and 450 nm (*2.2.25*), the solution shows 2 absorption maxima, at 257 nm and 358 nm. The specific absorbance at the maximum at 358 nm is 305 to 330, calculated with reference to the anhydrous substance.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *rutoside trihydrate CRS*.

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

Test solution. Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution. Dissolve 25 mg of *rutoside trihydrate CRS* in *methanol R* and dilute to 10.0 ml with the same solvent.

Plate: *TLC silica gel G plate R*.

Mobile phase: *butanol R, anhydrous acetic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (5:10:10:30:50 *V/V/V/V/V*).

Application: 10 µl.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with a mixture of 7.5 ml of a 10 g/l solution of *potassium ferricyanide R* and 2.5 ml of *ferric chloride solution R1* and examine for 10 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 the reference solution.

D. Dissolve 10 mg in 5 ml of *alcohol R*, add 1 g of *zinc R* and 2 ml of *hydrochloric acid R1*. A red colour develops.

TESTS

Light absorbing impurities (*2.2.25*): maximum 0.10 at wavelengths between 450 nm and 800 nm.

Dissolve 0.200 g in 40 ml of *2-propanol R*. Stir for 15 min, dilute to 50.0 ml with *2-propanol R* and filter.

Substances insoluble in methanol: maximum 3 per cent.

Shake 2.5 g for 15 min in 50 ml of *methanol R* at 20-25 °C. Filter under reduced pressure through a sintered-glass filter (1.6) (*2.1.2*) previously dried for 15 min at 100-105 °C, allowed to cool in a desiccator and tared. Wash the filter 3 times with 20 ml of *methanol R*. Dry the filter for 30 min at 100-105 °C. Allow to cool and weigh. The residue weighs a maximum of 75 mg.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.10 g of the substance to be examined in 20 ml of *methanol R* and dilute to 100.0 ml with mobile phase B.

Reference solution (a). Dissolve 10.0 mg of *rutoside trihydrate CRS* in 10.0 ml of *methanol R*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with mobile phase B.

Column:

– *size*: *l* = 0.25 m, Ø = 4.0 mm,