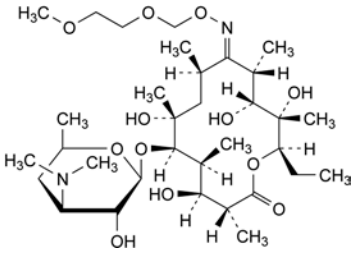
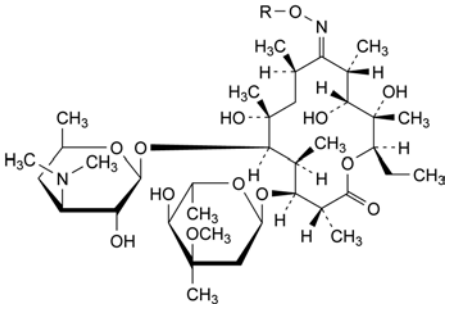
(2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *K*.

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],

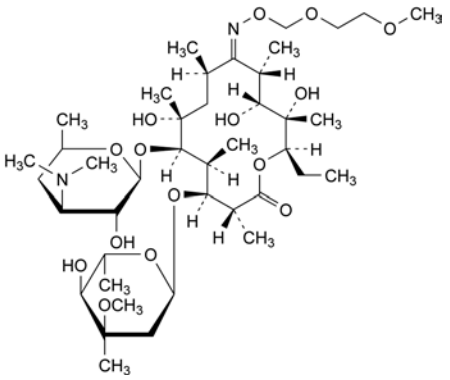


C. R = H: erythromycin 9-(*E*)-oxime,

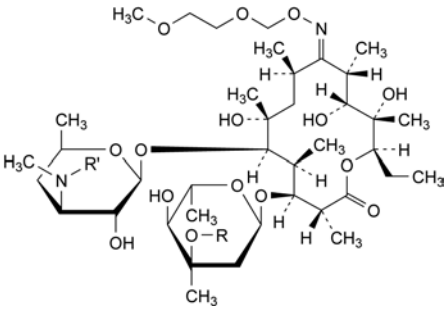
G. R = CH_2-O-CH_2-O-CH_2-CH_2-OCH_3: erythromycin 9-(*E*)-[*O*-[[(2-methoxyethoxy)methoxy]methyl]oxime],

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],

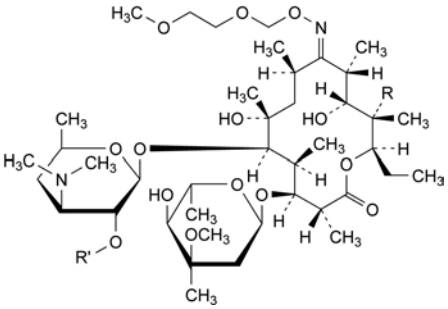


D. erythromycin 9-(*Z*)-[*O*-[(2-methoxyethoxy)methyl]oxime],



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].

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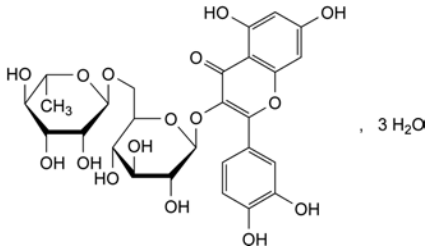
corrected 7.0

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 (96 per cent), 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 *ethanol (96 per cent) 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,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 30 °C.

Mobile phase:

- *mobile phase A*: mix 5 volumes of *tetrahydrofuran R* with 95 volumes of a 15.6 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*,
- *mobile phase B*: mix 40 volumes of *tetrahydrofuran R* with 60 volumes of a 15.6 g/L solution of *sodium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	50 → 0	50 → 100
10 - 15	0	100

Flow rate: 1 mL/min.

Detection: spectrophotometer at 280 nm.

Injection: 20 µL.

Relative retention with reference to rutoside (retention time = about 7 min): impurity B = about 1.1; impurity A = about 1.2; impurity C = about 2.5.

System suitability: reference solution (a):

- *peak-to-valley ratio*: minimum 10, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to rutoside.

Limits: locate the impurities by comparison with the chromatogram provided with *rutoside trihydrate CRS*:

- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.8; impurity C = 0.5,
- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
- *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent),
- *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Water (*2.5.12*): 7.5 per cent to 9.5 per cent, determined on 0.100 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

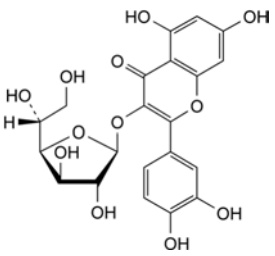
Dissolve 0.200 g in 20 mL of *dimethylformamide R*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M tetrabutylammonium hydroxide* is equivalent to 30.53 mg of $C_{27}H_{30}O_{16}$.

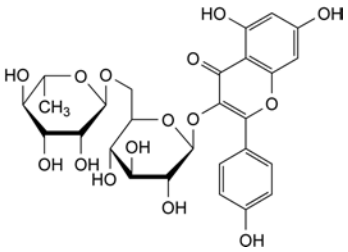
STORAGE

Protected from light.

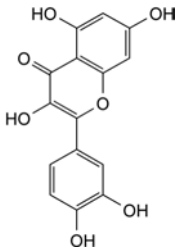
IMPURITIES



A. 2-(3,4-dihydroxyphenyl)-3-(β-D-glucofuranosyloxy)-5,7-dihydroxy-4*H*-1-benzopyran-4-one (isoquercitroside),



B. 3-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (kaempferol 3-rutinoside),



C. 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one (quercetin).