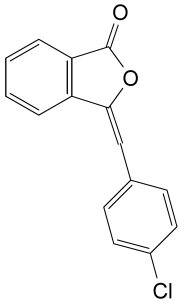
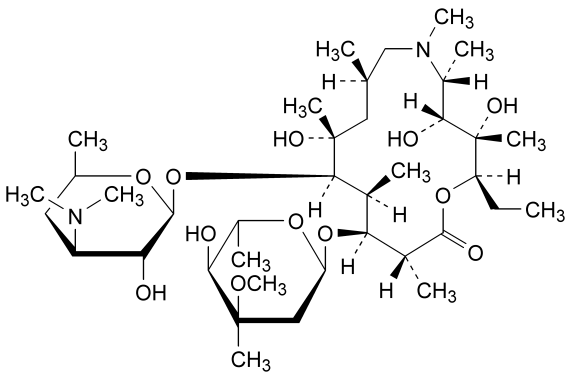


E. 3-(4-chlorobenzylidene)isobenzofuran-1(3*H*)-one.

01/2005:1649
corrected

AZITHROMYCIN

Azithromycinum



$C_{38}H_{72}N_2O_{12}$ M_r 749

DEFINITION

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

Content: 94.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, freely soluble in ethanol and in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *azithromycin CRS*.

If the spectra obtained show 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 test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

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

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

pH (*2.2.3*): 9.0 to 11.0.

Dissolve 0.100 g in 25.0 ml of *methanol R* and dilute to 50.0 ml with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): −45 to −49 (anhydrous substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture: *acetonitrile R, water R* (40:60 *V/V*).

Test solution (a). Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Test solution (b). Dilute 5.0 ml of test solution (a) to 20.0 ml with the solvent mixture.

Reference solution (a). Dissolve 50.0 mg of *azithromycin CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). Dissolve 5.0 mg of *azithromycin CRS* and 5.0 mg of *azithromycin impurity A CRS* in the solvent mixture and dilute to 50 ml with the solvent mixture.

Reference solution (d). Dissolve 2 mg of *azithromycin impurity B CRS* in the solvent mixture and dilute to 50 ml with the solvent mixture. Use this solution only for identification of the peak due to impurity B.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *end-capped polar-embedded octadecylsilyl amorphous organosilica polymer R* (5 µm),
– *temperature*: 70 °C.

Mobile phase: mix 10 volumes of a 34.84 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 6.5 with *phosphoric acid R*, 35 volumes of *acetonitrile R* and 55 volumes of *water R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 215 nm.

Injection: 100 µl; inject test solution (a) and reference solutions (b), (c) and (d).

Run time: 4.5 times the retention time of azithromycin.

Relative retention with reference to azithromycin (retention time = about 26 min): impurity D = about 0.37; impurity J = about 0.39; impurity A = about 0.42; impurity I = about 0.5; impurity C = about 0.65; impurity K = about 0.9; impurity F = about 1.6; impurity B = about 1.7; impurity G = about 2.8.

System suitability: reference solution (c):

– *resolution*: minimum 7.0 between the peaks due to impurity A and azithromycin.

Limits:

– *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent),
– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*): maximum 25 ppm.

Dissolve 2.0 g in a mixture of 15 volumes of *water R* and 85 volumes of *ethanol R* and dilute to 20 ml with the same mixture of solvents. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (2.5 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 *ethanol R*.

Water (*2.5.12*): 1.8 per cent to 6.5 per cent, determined on 0.20 g.

Sulphated ash (*2.4.14*): maximum 0.2 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: 25 µl; inject test solution (b) and reference solution (a).

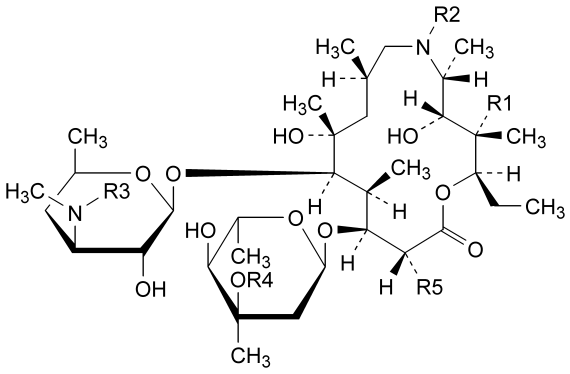
Calculate the percentage content of $C_{38}H_{72}N_2O_{12}$ using the declared content of *azithromycin CRS*.

STORAGE

In an airtight container.

IMPURITIES

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



A. R1 = OH, R2 = H, R3 = R4 = R5 = CH_3: 6-demethylazithromycin,

B. R1 = H, R2 = R3 = R4 = R5 = CH_3: 3-deoxyazithromycin (azithromycin B),

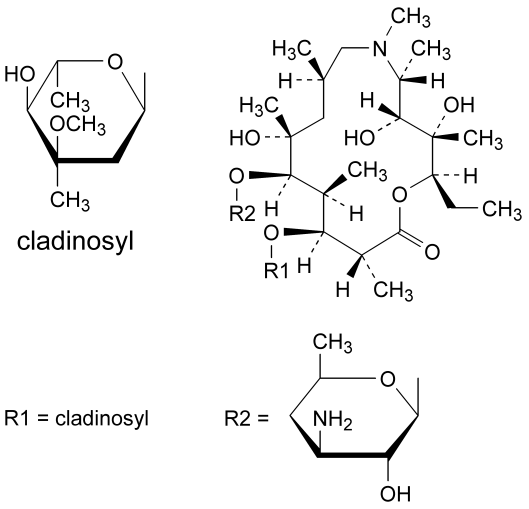
C. R1 = OH, R2 = R3 = R5 = CH_3, R4 = H: 3′-*O*-demethylazithromycin (azithromycin C),

D. R1 = OH, R2 = R3 = R4 = CH_3, R5 = CH_2OH: 14-demethyl-14-(hydroxymethyl)azithromycin (azithromycin F),

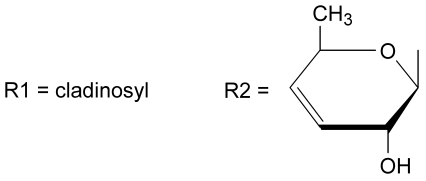
F. R1 = OH, R2 = R4 = R5 = CH_3, R3 = CHO: 3′-*N*-demethyl-3′-*N*-formylazithromycin,

G. R1 = OH, R2 = R4 = R5 = CH_3, R3 = SO_2-C_6H_4-CH_3: 3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulphonyl]azithromycin,

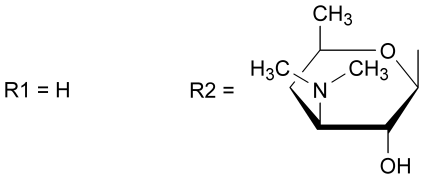
I. R1 = OH, R2 = R4 = R5 = CH_3, R3 = H: 3′-*N*-demethylazithromycin,



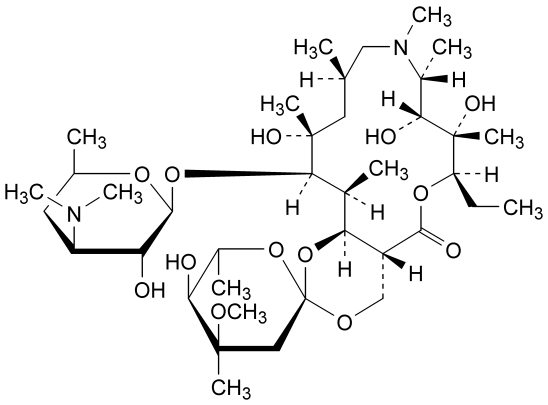
E. 3′-(*N*,*N*-didemethyl)azithromycin (aminoazithromycin),



H. 3′-de(dimethylamino)-3′,4′-didehydroazithromycin,



J. decladinosylazithromycin,



K. (2*S*,4′*R*,4a*R*,5′*S*,6′*S*,7*R*,8*S*,9*R*,10*R*,13*R*,15*R*,16*R*,17*S*,17a*S*)-7-ethyl-5′,8,9,15-tetrahydroxy-4′-methoxy-4′,6′,8,10,11,13,15,17-octamethyl-16-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]octadecahydro-5*H*-spiro[1,3-dioxino[4,5-*m*][1,6]oxazacyclopentadecine-2,2′-[2*H*]pyran]-5-one (azithromycin E).