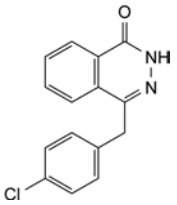
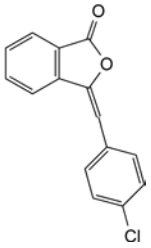


D. 4-(4-chlorobenzyl)phthalazin-1(2*H*)-one,

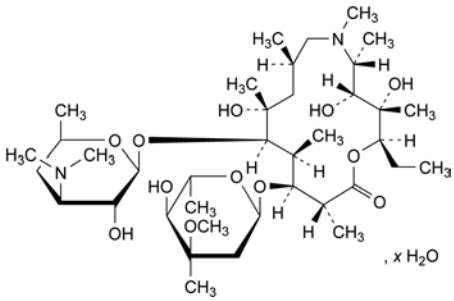


E. 3-(4-chlorobenzylidene)isobenzofuran-1(3*H*)-one.

01/2011:1649

AZITHROMYCIN

Azithromycinum



$C_{38}H_{72}N_2O_{12},xH_2O$ M_r 749 (anhydrous substance)
with x = 1 or 2

Azithromycin monohydrate: [121470-24-4]
Azithromycin dihydrate: [117772-70-0]

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. The degree of hydration is 1 or 2.

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 powder.

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

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *azithromycin CRS*.

If the spectra obtained in the solid state show differences, prepare further spectra using 90 g/L solutions in *methylene chloride R*.

TESTS

Solution S. Dissolve 0.500 g in *anhydrous 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*).

Solvent mixture. Prepare a 1.73 g/L solution of *ammonium dihydrogen phosphate R* adjusted to pH 10.0 with *ammonia R*. Transfer 350 mL of this solution to a suitable container. Add 300 mL of *acetonitrile R1* and 350 mL of *methanol R1*. Mix well.

Test solution. Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

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

Reference solution (b). Dissolve the contents of a vial of *azithromycin for system suitability CRS* (containing impurities F, H and J) in 1.0 mL of the solvent mixture and sonicate for 5 min.

Reference solution (c). Dissolve 8.0 mg of *azithromycin for peak identification CRS* (containing impurities A, B, C, E, F, G, I, J, L, M, N, O and P) in 1.0 mL of the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl amorphous organosilica polymer for mass spectrometry R* (5 µm);
– *temperature*: 60 °C.

Mobile phase:

– *mobile phase A*: 1.80 g/L solution of *anhydrous disodium hydrogen phosphate R* adjusted to pH 8.9 with *dilute phosphoric acid R* or with *dilute sodium hydroxide solution R*;
– *mobile phase B*: *methanol R1, acetonitrile R1* (250:750 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	50 → 45	50 → 55
25 - 30	45 → 40	55 → 60
30 - 80	40 → 25	60 → 75
80 - 81	25 → 50	75 → 50
81 - 93	50	50

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 50 µL.

Identification of impurities: use the chromatogram supplied with *azithromycin for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, E, F, G, I, J, L, M, N, O and P; use the chromatogram supplied with *azithromycin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity H.

Relative retention with reference to azithromycin (retention time = 45-50 min): impurity L = about 0.29; impurity M = about 0.37; impurity E = about 0.43; impurity F = about 0.51; impurity D = about 0.54; impurity J = about 0.54; impurity I = about 0.61; impurity C = about 0.73; impurity N = about 0.76; impurity H = about 0.79; impurity A = about 0.83; impurity P = about 0.92; impurity O = about 1.23; impurity G = about 1.26; impurity B = about 1.31.

System suitability: reference solution (b):

– *peak-to-valley ratio*: minimum 1.4, where H_p = height above the baseline of the peak due to impurity J and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity F.

Limits:

– *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity F = 0.3; impurity G = 0.2; impurity H = 0.1; impurity L = 2.3; impurity M = 0.6; impurity N = 0.7;

– *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent);

– *impurities A, C, E, F, H, I, L, M, N, O, P*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *sum of impurities D and J*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *impurity G*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

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

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

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard the peaks eluting before impurity L and after impurity B.

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 *anhydrous ethanol R* and dilute to 20 mL with the same mixture of solvents. 12 mL of the solution complies with test B. Prepare the reference solution 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 *anhydrous ethanol R*.

Water (*2.5.12*): 1.8 per cent to 6.5 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solution A. Mix 60 volumes of *acetonitrile R1* and 40 volumes of a 6.7 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 8.0 with *phosphoric acid R*.

Test solution. Dissolve 53.0 mg of the substance to be examined in 2 mL of *acetonitrile R1* and dilute to 100.0 mL with solution A.

Reference solution (a). Dissolve 53.0 mg of *azithromycin CRS* in 2 mL of *acetonitrile R1* and dilute to 100.0 mL with solution A.

Reference solution (b). Dissolve 5 mg of the substance to be examined and 5 mg of *azithromycin impurity A CRS* in 0.5 mL of *acetonitrile R1* and dilute to 10 mL with solution A.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl vinyl polymer for chromatography R* (5 µm);

– *temperature*: 40 °C.

Mobile phase: mix 60 volumes of *acetonitrile R1* and 40 volumes of a 6.7 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 11.0 with a 560 g/L solution of *potassium hydroxide R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 10 µL.

Run time: 1.5 times the retention time of azithromycin.

Retention time: azithromycin = about 10 min.

System suitability: reference solution (b):

– *resolution*: minimum 3.0 between the peaks due to impurity A and azithromycin.

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

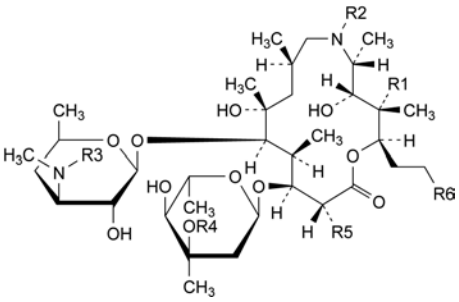
STORAGE

In an airtight container.

IMPURITIES

Specified impurities: *A, B, C, D, E, F, G, H, I, J, L, M, N, O, P.*

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. R1 = OH, R2 = R6 = H, R3 = R4 = R5 = CH_3: 6-demethylazithromycin,

B. R1 = R6 = H, R2 = R3 = R4 = R5 = CH_3: 3-deoxyazithromycin (azithromycin B),

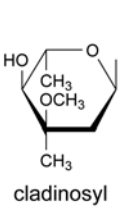
C. R1 = OH, R2 = R3 = R5 = CH_3, R4 = R6 = H: 3″-*O*-demethylazithromycin (azithromycin C),

D. R1 = OH, R2 = R3 = R4 = CH_3, R5 = CH_2OH, R6 = H: 14-demethyl-14-(hydroxymethyl)azithromycin (azithromycin F),

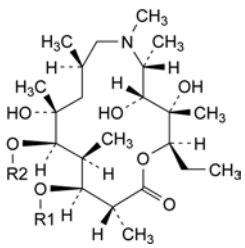
F. R1 = OH, R2 = R4 = R5 = CH_3, R3 = CHO, R6 = H: 3′-*N*-demethyl-3′-*N*-formylazithromycin,

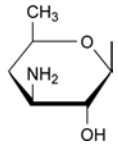
I. R1 = OH, R2 = R4 = R5 = CH_3, R3 = R6 = H: 3′-*N*-demethylazithromycin,

O. R1 = OH, R2 = R3 = R4 = R5 = R6 = CH_3: 2-desethyl-2-propylazithromycin,

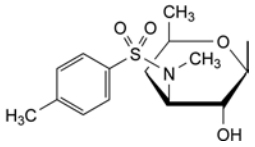


cladinosyl

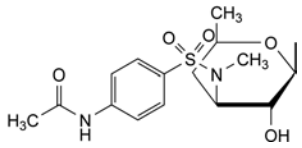


R1 = cladinosyl R2 = 

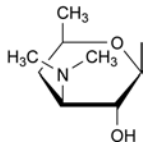
E. 3′-(*N*,*N*-didemethyl)azithromycin (aminoazithromycin),

R1 = cladinosyl R2 = 

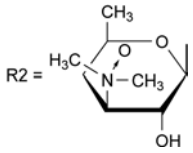
G. 3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]azithromycin,

R1 = cladinosyl R2 = 

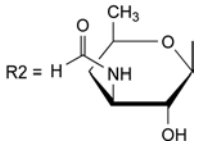
H. 3′-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3′-*N*-demethylazithromycin,

R1 = H R2 = 

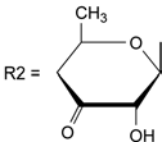
J. 13-*O*-decladinosylazithromycin,

R1 = cladinosyl R2 = 

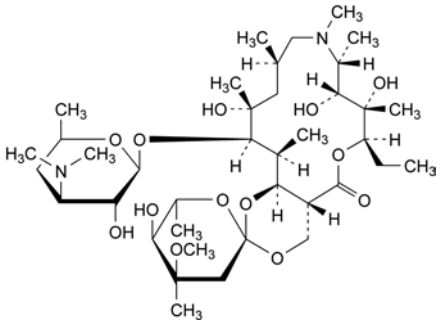
L. azithromycin 3′-*N*-oxide,

R1 = cladinosyl R2 = 

M. 3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin,

R1 = cladinosyl R2 = 

N. 3′-de(dimethylamino)-3′-oxoazithromycin,



K. C^{14},1″-epoxyazithromycin (azithromycin E),

P. unknown structure.