



Azithromycin

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BACKGROUND

A team of researchers at the Croatian pharmaceutical company Pliva, led by Dr. Slobodan Đokić, discovered azithromycin in 1980. It was patented in 1981. Pfizer launched azithromycin under Pliva's license in other markets under the brand name Zithromax in 1991. After several years, the U.S. Food and Drug Administration approved AzaSite, an ophthalmic formulation of azithromycin, for the treatment of eye infections [1].

1. DESCRIPTION

1.1. Nomenclature

1.1.1 Systematic chemical names [2–5]

(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*-3-*O*-dimethyl- α -*L*-ribo-hexopyranosyloxy)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-(3,4,6-trideoxy-3-dimethylamino- β -*D*-xylo-hexopyranosyloxy)-1-oxa-6-aza-cyclopentadecan-15-one dehydrate.

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xylo-]oxy}-1-oxa-6-azacyclopentadec-13-yl 2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranoside.

(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.

(2*R*,3*S*,4*R*,5*R*,8*R*,11*R*,13*S*,14*R*)-11-(((2*S*,3*S*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-2-ethyl-3,4,10-trihydroxy-13-(((2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2*H*-pran-2-yl)oxy)-3,5,6,8,10,14-hexamethyl-1-oxa-6-azacyclopentadecan-15-one.

N-methyl-11-aza-10-deoxo-10-dihydroerythromycin A; 9-deoxo-9a-methyl-9a-aza-homoerythromycin A; CP-62993; XZ-450; Azitrocin; Sumamed; Trozocina; Zithromax; Zitromax.

1.1.2 Nonproprietary names

Generic [6]: azithromycin

Synonyms [2–7]: Atsitromysiini; Azithromycine; Azithromycinum; Azitromicina; Azitromicinas; Azitromisin; Azitromycin; Azytromycyna; CP-62993; XZ-450; Azithramycine; Azithromycin Dihydrate; Azithromycine [French]; Azithromycinum [Latin]; Azitromicina [Spanish].

1.1.3 Proprietary names [3,7,8]

Azacid[®] (Biosen: TR); Azitromin[®] (Farmasa: BE); Azitromax[®] (Pfizer: NO); Azro[®] (Eczacibasi: TR); Cronopen[®] (Elea: AR); Hemomycin[®] (Hemofarm: YU); Misultina[®] (Microsules: AR); Mixoterin[®] (Roux-Ocefa: AR); Sumamed[®] (Pliva: CZ, HR, PL); Triamid[®] (Beta: AR); Zifin[®] (Pratapa: ID); Zithrax[®] (Dankos: ID); Zithromax[®] (Pfizer: NL); Zitromax[®] (Pfizer: AR, BE, LU, TR); Zitrotek[®] (Pfizer: TR);

Dihydrate: Arzomicin[®] (Sintyal: AR); Azadose[®] (Pfizer: FR); Azatek[®] (Biosen: TR); Azithral[®] (Alembic: IN); Azitrin[®] (Andromaco: AR); Azitro[®] (Deva: TR); Azitrocin[®] (Pfizer: MX); Azitrocin[®] (Roerig: IT); Azitromax[®] (Pfizer: NO,SE); Azitromicina Richet[®] (Richet: AR); Azitrotek[®] (Deva: TR); Aziwok[®] (Wockhardt: IN); Azomax[®] (Kocak: TR); Azro[®] (Eczacibasi: TR); Cronopen[®] (Elea: AR); Goxal[®] (Pharmacia: ES); Ribotrex[®] (Pierre Fabre: IT); Toraseptol[®] (Lesvi: ES); Triamid[®] (Beta: AR); Trozocina[®] (Sigma-Tau: IT); Ultreon[®] (Pfizer: DE); Vinzam[®] (Funk: ES); Zentavion[®] (Vita: ES); Zistic[®] (Bernofarm: ID); Zithromax[®] (Bayer: DE); Zithromax[®] (Mack: DE); Zithromax[®] (Pfizer: AT, AU, CA, CH, FI, FR, ID, IE, IN, PT, UK, US); Zitromax[®] (Pfizer: BE, BR, DK, ES, IT).

1.2. Formulae [2,3]

1.2.1 Empirical formula, molecular weight, CAS number

	Empirical formula	Molecular weight	CAS number
Anhydrous azithromycin	C ₃₈ H ₇₂ N ₂ O ₁₂	748.984	83905-01-5
Monohydrate azithromycin	C ₃₈ H ₇₂ N ₂ O ₁₂ H ₂ O	767.02	121479-24-4
Azithromycin dihydrate	C ₃₈ H ₇₂ N ₂ O ₁₂ 2H ₂ O	785.0	117772-70-0

1.2.2 Structural formula

See Figure 1.1

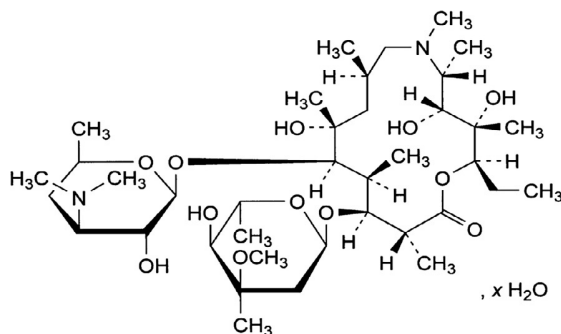


Figure 1.1 Structural formula of azithromycin hydrate.

1.3. Elemental analysis

The theoretical elemental composition of azithromycin is as follows [5]:

Carbon: 60.94 %; Hydrogen: 9.69%; Nitrogen: 3.74%; Oxygen: 25.63%

1.4. Appearance [2]

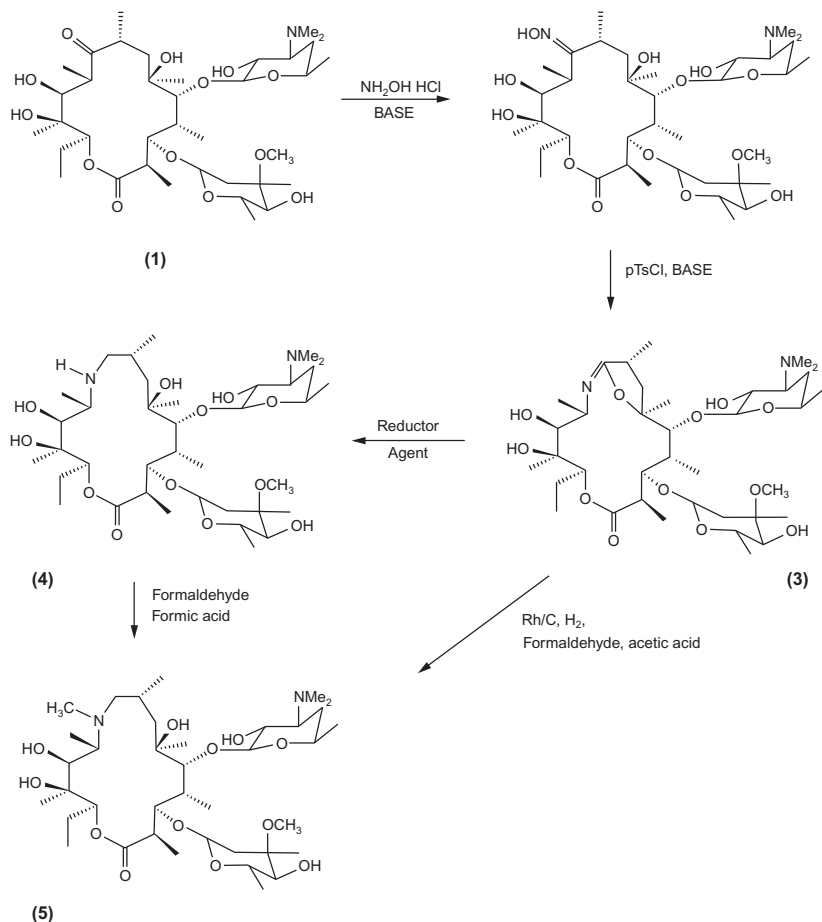
A white or almost white powder.



2. METHODS OF PREPARATION OF AZITHROMYCIN

Azithromycin (**5**) was prepared from erythromycin A [9,10] by treating the erythromycin (**1**) in methanol with hydroxylamine hydrochloride and a base at reflux temperature for 10 h to form oxime (**2**). The oxime was isolated, purified, and subjected to Beckmann's rearrangement to obtain the intermediary (6,9-iminoether) (**3**) (Scheme 1.1) in aqueous acetone in the presence of *p*-toluenesulfonyl chloride and base for 2 h at 5 °C (and 2 h more at room temperature). The iminoether was reduced to the secondary amine (**4**) with sodium borohydride in methanol [11,12] or by catalytic hydrogenation in the presence of platinum dioxide and acetic acid as solvents [13].

Another alternate synthetic method for azithromycin is reported [14] The iminoether (**3**) was prepared in single step (Scheme 1.2) from erythromycin A (**1**), by treating the erythromycin A (**1**) solution in acetone with *O*-mesitylene-sulfonylhydroxylamine, to form the mesitylenesulfonyloxime “*in situ*” from erythromycin A, which was treated with an aqueous base (sodium bicarbonate) at 0 °C, and then the intermediary 6-9-iminoether (**3**) (Scheme 1.2) was produced by a Beckmann's rearrangement. The iminoether (**3**) was reduced with reductive methylation using common techniques [15] to obtain azithromycin (**5**).



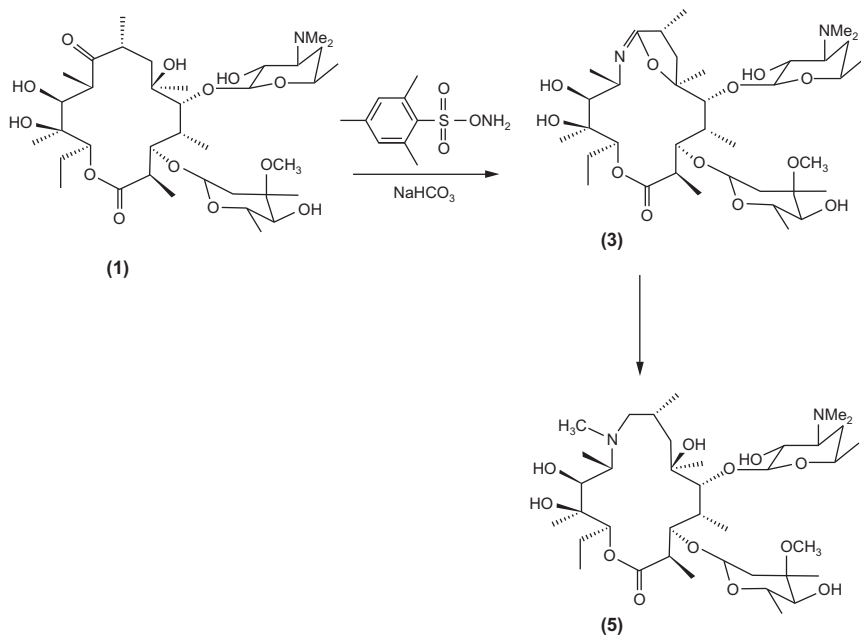
Scheme 1.1 Beckmann's rearrangement to obtain the intermediary (6,9-iminoether) for preparation of Azithromycin.

Azithromycin is a semisynthetic 15-membered macrolide antibiotic, which is derived from erythromycin A by a sequence of oximation, Beckmann rearrangement, reduction, and N-methylation [16–19]. Rengaraju *et al.* [20] improved process for preparing nonhygroscopic azithromycin dehydrate.

3. PHYSICAL CHARACTERISTICS

3.1. Specific optical rotation [5,21]

$[\alpha]^{20} -45^\circ$ to -49° (anhydrous substance) ($C=1$ in anhydrous ethanol R)
 $[\alpha]^{20} -37^\circ$ ($C=1$ in CHCl_3)



Scheme 1.2 An alternate synthetic method for Azithromycin.

3.2. Ionization constant [22]

$$\text{p}K_{\text{a}} = 7.34$$

3.3. Solubility characteristics [21]

Azithromycin is practically insoluble in water and freely soluble in anhydrous ethanol and methylene chloride.

3.4. Partition coefficient

The octanol/water partition coefficient (K_{ow}) of azithromycin was 0.65 at 20 °C and pH 7 [23]. Adsorption isotherm studies indicated that the thermodynamic data revealed that the adsorption of azithromycin on the surface of zinc was endothermic, spontaneous, and consistent with the adsorption model of Langmuir [24].

3.5. Particle morphology

Gandhi *et al.* [25] viewed the commercial sample, dehydration and monohydration of azithromycin by scanning electron microscopy (Jeol electron

microscope, D-6000, Japan). The samples were sputter coated with gold before examination, and they found that the internal crystal structure appears to be the same, as is evident from the similar enthalpy of fusion for all three samples [26].

3.6. Crystallographic properties

3.6.1 Single crystal structure

Data were collected at room temperature using Bruker X-ray diffractometers equipped with copper radiation and graphite monochromators. Structures were solved using direct methods. The SHELXTL computer library provided by Bruker AXS, Inc. facilitated all necessary crystallographic computations and molecular displays (SHELXTL™ Reference Manual, Version 5.1, Bruker AXS, Madison, WI, USA) [14].

The molecular structure of the anhydrous crystalline azithromycin obtained is shown in Figure 1.2, and the structure was elucidated by single crystal X-ray diffraction, finding that it coincides with the anhydrous crystalline form, with a tetragonal crystal system and the space group $P4_22_12$. These and other crystallographic data from the diffraction analysis are compared with data reported for the dihydrated crystalline form. A displays peaks at 9.3, 13.0 and 18.7 degrees of 2-theta [27].

3.6.2 X-ray powder diffraction pattern [28]

The X-ray powder diffraction pattern of azithromycin has been measured using a Bruker D5000 diffractometer (Madison, Wis.) equipped with copper radiation, fixed slits (1.0, 1.0, 0.6 mm), and a Kevex solid state detector. The pattern obtained is shown in Figure 1.3, and Azithromycin dehydrate displays peaks at 7.2, 7.9, 9.3, 9.9, 11.2, 12.0, 12.7, 13.0, 14.0, 15.6, 16.0, 16.4, 16.8, 17.5, 18.2, 18.7, 19.1, 19.8, 20.5, 20.9, 21.2, 21.6, 21.8, and 24.0, the data was collected from 3.0 to 40.0 degrees in 2-theta using a step size of 0.04 degrees and a step time of 1.0 seconds.

3.7. Hygroscopicity

Azithromycin was found to exhibit pseudopolymorphism and can exist as monohydrate and dihydrate. The anhydrous form of AZI seemed to be unstable since it converted to dihydrate on storage at room temperature. On the other hand, monohydrate in the presence of moisture can convert

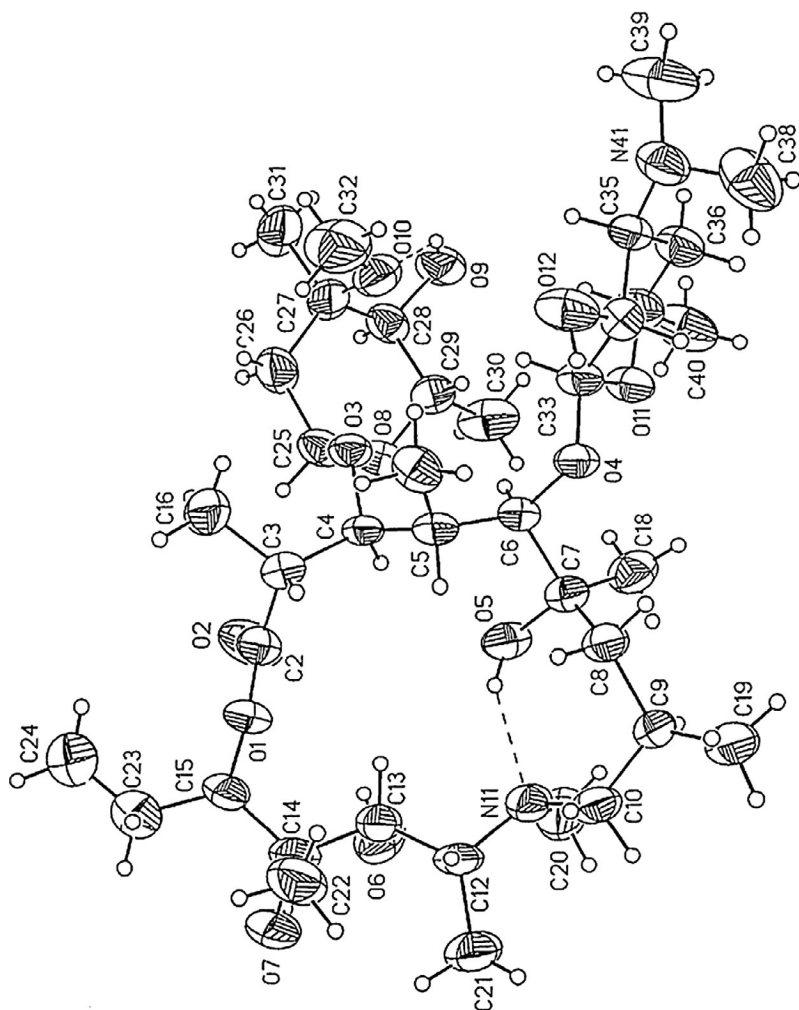


Figure 1.2 An X-ray crystal structure of the crystalline anhydrous azithromycin.

to the more stable dihydrate form. Therefore, the most stable form of AZI is dehydrate [25].

Allen *et al.* [29] found that azithromycin was obtained as hygroscopic monohydrate when it was prepared by crystallization from ethanol and water, and nonhygroscopic dehydrate azithromycin was prepared by crystallization from tetrahydrofuran and aliphatic (C_5 – C_7) hydrocarbon in the presence of at least two molar equivalents of water.

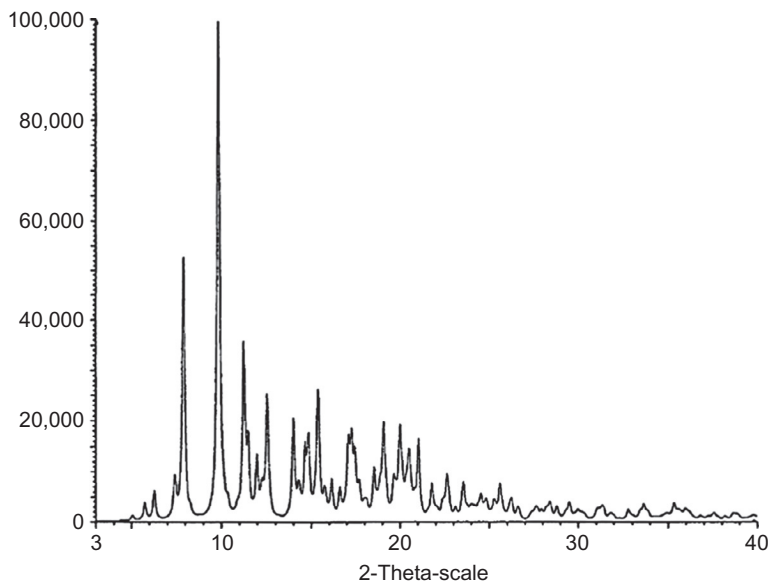


Figure 1.3 An experimental powder X-ray diffraction pattern of azithromycin dehydrate [8].

3.8. Thermal methods of analysis

3.8.1 Melting behavior

The melting range of azithromycin crystals is between 113 and 115 °C [5].

3.8.2 Differential scanning calorimetry [25]

The differential scanning calorimetry (DSC) thermogram of azithromycin was recorded on a DSC (Mettler, Toledo DSC 821 Switzerland) using Mettler Star system. The curve shown in Figure 1.4 was collected from 0 to 250 °C with nitrogen purging (80 ml/min), and using a heating rate of 10 °C/min. DSC thermograms of anhydrous, dehydrate, and monohydrate are shown in Figure 1.4, and the thermal characteristics are shown in Table 1.1.

3.8.3 Thermogravimetric analysis

Thermogravimetric analysis was carried out using TGA (Mettler, LMI JM Switzerland) apparatus. The samples (1–5 mg) were placed in aluminum pans and heated up to 250 °C at a rate of 1 °C/min under nitrogen purge (40 ml/min). The TGA thermogram obtained with the AZC sample is given in Figure 1.5. This thermogram exhibited the transition from the dihydrate to the anhydrous form of azithromycin. The observed weight loss

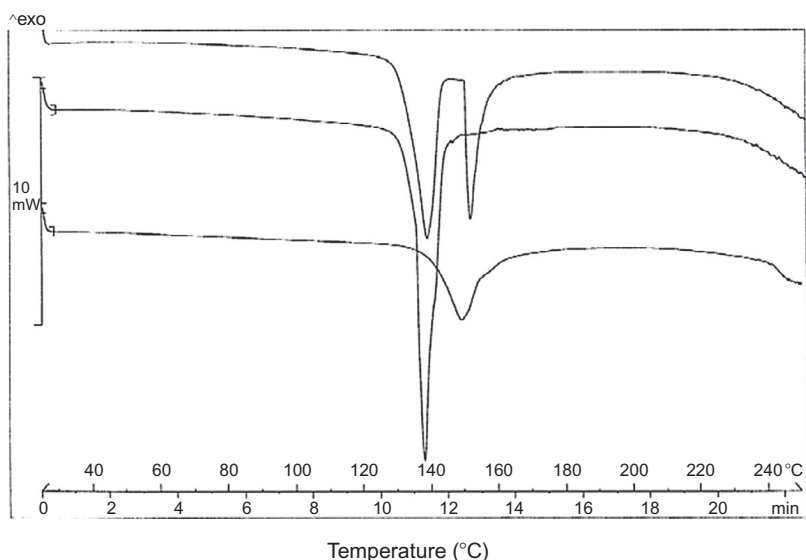


Figure 1.4 DSC thermograms of different forms of AZI. Upper curve depicts the commercial sample of AZI; middle curve shows the endotherm of dihydrate (DH) and lower curve shows the endotherm of monohydrate (MH). The thermograms were generated using a sealed pan.

Table 1.1 Thermal analysis and KFT of different forms of AZI

Sample name	DSC analysis		Water content	
	Temperature range (°C)	Heat fusion (J/g)	TGA (%)	KFT (%)
<i>Anhydrous azithromycin</i>	132.29–143.09	62.73 ± 7.13	4.452 ± 0.18	4.57 ± 0.03
	149.83–155.48	30.41 ± 2.93		
<i>Monohydrate azithromycin</i>	134.65–155.48	92.99 ± 8.58	4.155 ± 0.41	4.35 ± 0.28
<i>azithromycin dihydrate</i>	139.88–156.31	92.83 ± 2.41	2.472 ± 0.41	2.39 ± 0.79

of 4.38% corresponded to the stoichiometric weight loss of two water molecules [30].

Gandhi *et al.* [25] were found a stoichiometric weight loss of two water molecules (theoretical weight loss 4.58%) for CS and DH by TGA, while MH showed a weight loss corresponding to one molecule of water (theoretical weight loss 2.29%) as shown in Figure 1.6. The results were in good

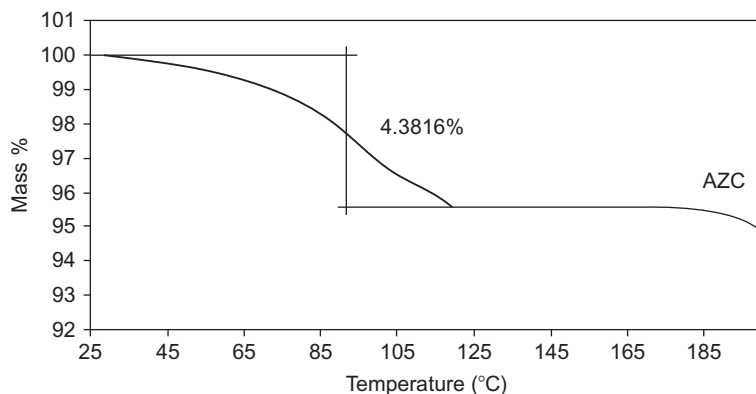


Figure 1.5 TGA thermogram of AZC.

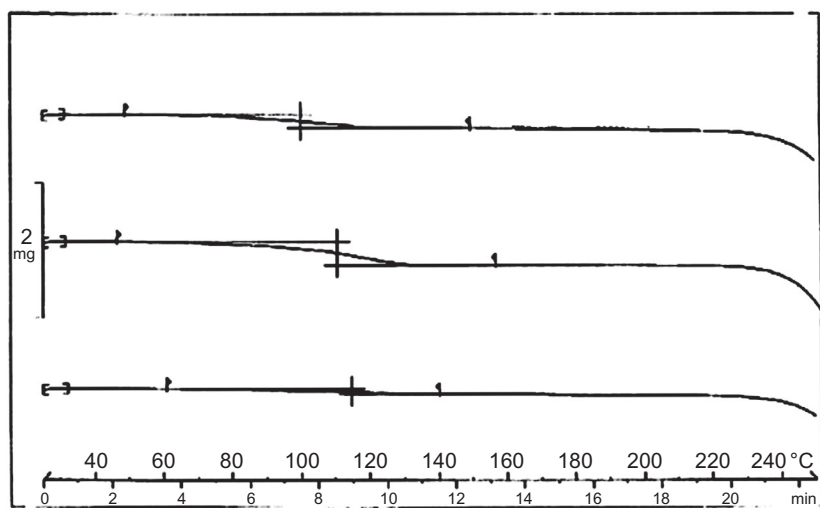


Figure 1.6 TGA thermograms of different forms of AZI. Upper curve indicates stoichiometric weight loss of two water molecules in CS, middle curve indicates weight loss of two water molecules in DH, and lower curve indicates weight loss of one water molecule in MH.

agreement with those found by Karl Fischer titration KFT as shown in Table 1.1.

3.8.4 Karl Fischer titration (KFT) [25]

Water content in different forms of AZI was determined using a Karl Fischer titrimeter (Metrohm, 716 DMS, Switzerland). Samples (20–25 mg) were

accurately weighed and quickly transferred to the titration vessel containing anhydrous methanol.

3.8.5 Boiling point, enthalpy of vapor, flash point, and vapor pressure

The calculated value of the boiling point of azithromycin under a pressure of 760 mmHg was 822.1 °C. The enthalpy of vapor calculated value was 135.99 KJ/mol. The value of flash point was found to be 451 °C, and the vapor pressure was calculated to be 251×10^{-31} mmHg at 25 °C [31].

3.9. Spectroscopy

3.9.1 UV/Vis spectroscopy

The ultraviolet spectrum of azithromycin dihydrate in methanol and mobile phase (methanol: acetonitrile: phosphate buffer pH 6.7: tetrahydrofuran, 15:25:60:2.5, v/v) shown in Figure 1.7. The figures were recorded using a double beam Model GBC 916UV VIS spectrophotometer (GBC Scientific Equipment Pty Ltd., Melbourne, Victoria, Australia). The values of wavelength maximum in nanometer ($\lambda_{\max}$) are 201.6 nm on methanol and 199.2 nm on mobile phase. The spectra of azithromycin in methanol (Figure 1.7A) and mobile phase (Figure 1.7B) were shown below.

3.9.2 Vibrational spectroscopy [32]

The infrared absorption spectrum of azithromycin is shown in Figure 1.8. It was obtained in a KBr disc using a FT-IR Nicolet® Imoact 410 instrument, infrared spectrophotometer. The principal peaks were 3561, 3496, 13344, 1282, 1269, 1251, and 1083.

3.9.3 Nuclear magnetic resonance spectrometry [33]

3.9.3.1 ¹H NMR spectrum [33]

The ¹H nuclear magnetic resonance (NMR) spectrum of AZI was recorded using an internal deuterium lock at ambient probe temperatures on the following instruments: Bruker DPX-400 (400 MHz), Bruker Avance DRX-400 (400 MHz), Bruker Avance 500 BB-ATM (500 MHz), and Bruker Avance 500 Cryo Ultrashield (500 MHz). An internal reference of δ_H 7.26 was used for the residual CHCl₃ in CDCl₃. Data are represented as follows: chemical shift (in ppm to the nearest 0.01 ppm), integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant (J in Hz to the nearest 1 Hz), and assignments, which were determined either on the basis of unambiguous chemical shift or coupling pattern,

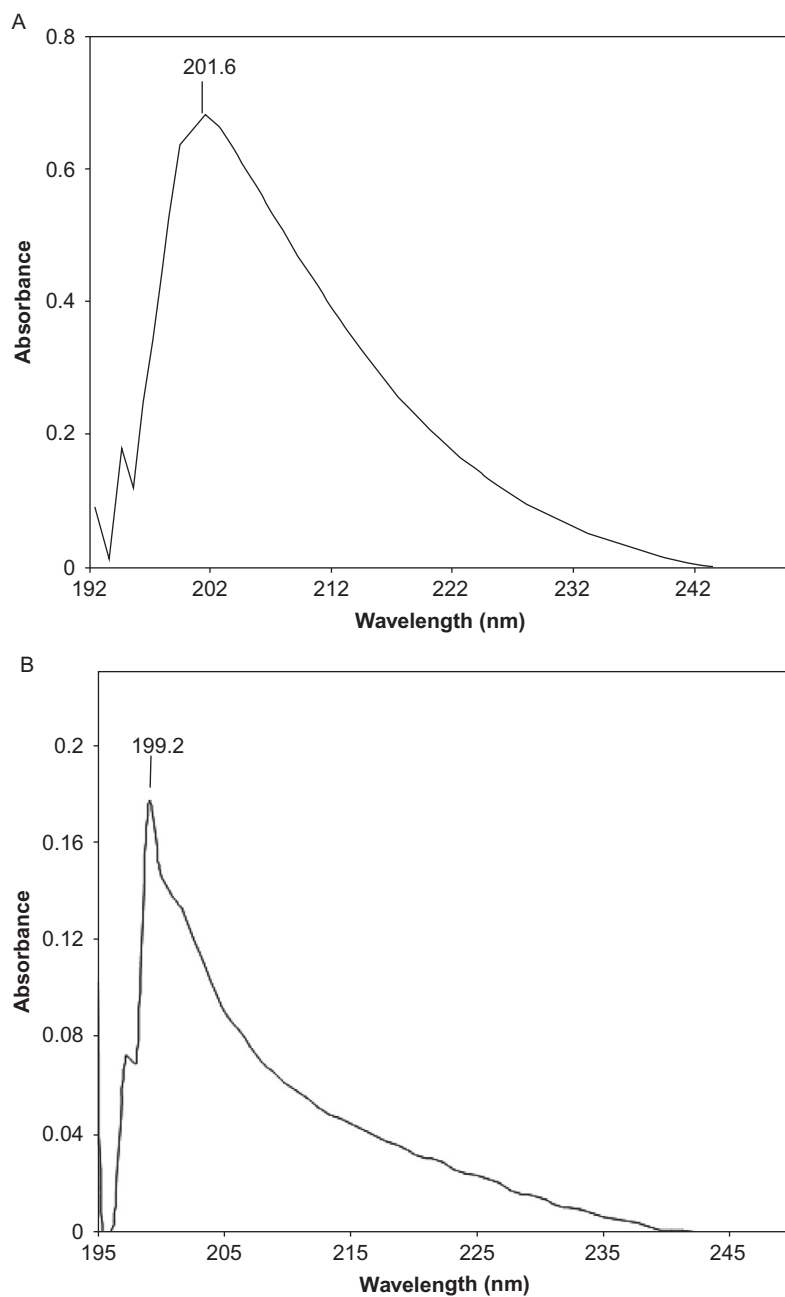


Figure 1.7 (A) The ultraviolet spectrum of AZI dihydrate in methanol. (B) The ultraviolet spectrum of AZI dihydrate in mobile phase.

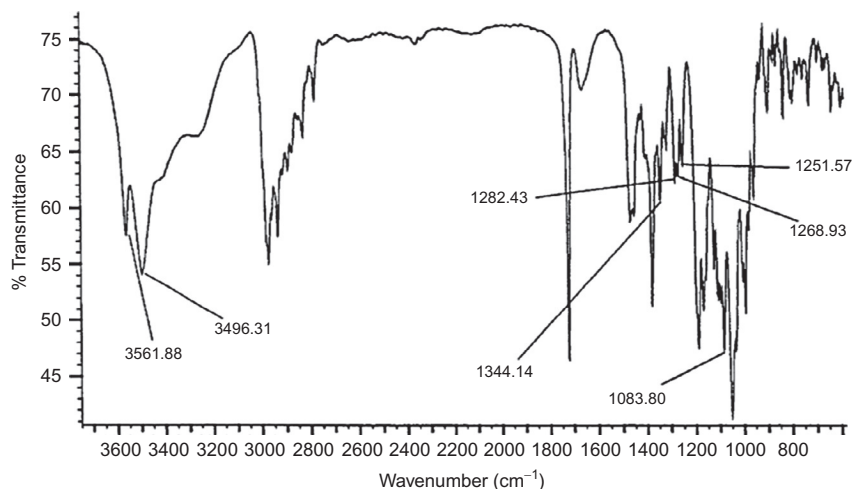


Figure 1.8 Infrared absorption spectrum of azithromycin.

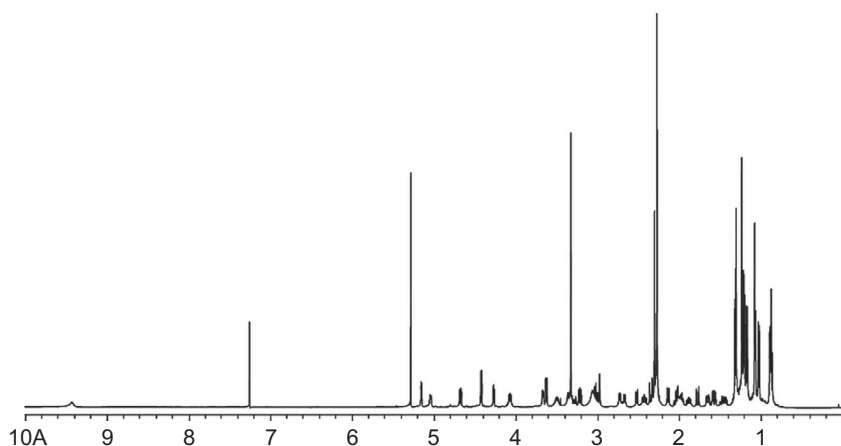


Figure 1.9 Full ¹H NMR spectrum of azithromycin in CDCl₃.

by patterns observed in 2D experiments (¹H-¹H COSY and HMQC) or by analogy to fully interpreted spectra for related compounds.

Azithromycin is shown in [Figure 1.9](#) (full ¹H NMR spectrum). The assignments for the observed bands are provided in [Table 1.2](#).

3.9.3.2 ¹³C NMR spectrum [\[33\]](#)

The ¹³C NMR spectrum of azithromycin was recorded by broadband proton spin decoupling at ambient probe temperature using an internal

Table 1.2 Assignments for the resonance bands observed in the ¹H NMR spectrum of azithromycin in CDCl₃
See [Figure 1.10 reference 33](#)

Chemical shift (ppm)	Number of protons	Multiplicity and coupling constant (J)	Assignment
9.44	1		(1H, C ₂ —OH)
5.13	1	Doublet (5 Hz)	C ₁ ''—H
4.74	1	Doublet (6 Hz)	C ₁₁ —OH
4.68	1	Doublet of doublets (10 Hz)	C ₁₃ —H
4.43	1	Doublet (7 Hz)	C ₁ '—H
4.29	1	Doublet of doublets (5, 2 Hz)	C ₃ —H
4.08	1	Doublet of quartet (10, 6 Hz)	C ₅ '—H
3.68	1	Doublet (5 Hz)	C ₁₁ —H
3.65	1	Doublet (7 Hz)	C ₅ —H
3.55–3.47	1	Multiple	C ₅ '—H
3.35	1	Singlet	C ₁₂ —OH
3.34	3	Singlet	C ₃ '—OCH ₃
3.23	1	Doublet of doublets (10, 7 Hz)	C ₂ '—H
3.03	1	app. triplet (10 Hz)	C ₄ '—H
2.89	1	Singlet	C ₆ —OH
2.78–2.72	1	Multiple	C ₂ —H
2.69	1	Quartet (7 Hz)	C ₁₀ —H
2.52	1	Doublet (10 Hz)	C ₉ —H
2.46–2.41		Multiple	C ₃ '—H
2.35	1	Doublet (15 Hz)	C ₂ '—H
2.31	3	Singlet	C ₁ —H ₃
2.28	6	Singlet	C ₃ '—N(CH ₃) ₂
2.15	1	Doublet (11 Hz)	C ₄ '—OH
2.07–1.95	2	Multiple	C ₄ —H and C ₈ —H

Continued

Table 1.2 Assignments for the resonance bands observed in the ^1H NMR spectrum of azithromycin in CDCl_3 —cont'd

Chemical shift (ppm)	Number of protons	Multiplicity and coupling constant (J)	Assignment
2.02	1	Doublet (10 Hz)	$\text{C}_9\text{—}\underline{\text{H}}$
1.93–1.85	1	Multiple	$\text{C}_{13}\text{—}\underline{\text{CH}_2}\text{—CH}_3$
1.79	1	Doublet (15 Hz)	$\text{C}_7\text{—H}$
1.67–1.63	1	Multiple	$\text{C}_4'\text{—}\underline{\text{H}}$
1.58	1	Doublet of doublets (15, 5 Hz)	$\text{C}_2''\text{—}\underline{\text{H}}$
1.51–1.43	1	Multiple	$\text{C}_{13}\text{—}\underline{\text{CH}_2}\text{—CH}_3$
1.32	3	Doublet (5 Hz)	$\text{C}_5''\text{—}\underline{\text{CH}_3}$
1.31	3	Singlet	$\text{C}_6\text{—}\underline{\text{CH}_3}$
1.24	3	Singlet	$\text{C}_3''\text{—}\underline{\text{CH}_3}$
1.26–1.21	2	Multiple	$\text{C}_7\text{—}\underline{\text{H}}$ and $\text{C}_4'\text{—}\underline{\text{H}}$
1.22	3	Doublet (6 Hz)	$\text{C}_5'\text{—}\underline{\text{CH}_3}$
1.19	3	Doublet (7 Hz)	$\text{C}_2\text{—}\underline{\text{CH}_3}$
1.08	1	Doublet (7 Hz)	$\text{C}_{10}\text{—}\underline{\text{CH}_3}$
1.08	3	Singlet	$\text{C}_{12}\text{—}\underline{\text{CH}_3}$
1.04	3	Doublet (7 Hz)	$\text{C}_4\text{—}\underline{\text{CH}_3}$
0.90	3	Doublet (7 Hz)	$\text{C}_8\text{—}\underline{\text{CH}_3}$
0.89	3	Triplet (7 Hz)	$\text{C}_{13}\text{—CH}_2\text{—}\underline{\text{CH}_3}$

deuterium lock. All chemical shift values are reported in ppm to the nearest 0.01 ppm. An internal reference of δ_{C} 77.0 was used for CDCl_3 . Assignments were supported by DEPT editing and determined either on the basis of unambiguous chemical shift, by patterns observed in 2D experiments (HMQC) or by analogy to fully interpreted spectra for related compounds.

Azithromycin is shown in [Figure 1.11](#) (full ^{13}C NMR spectrum). The assignments for the observed bands are provided in [Table 1.3](#), which are consistent with the 13 carbon contents of azithromycin.

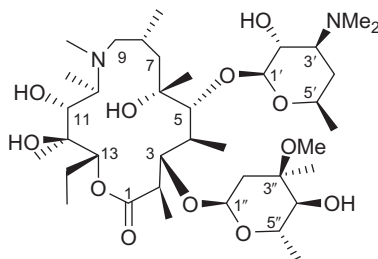


Figure 1.10 AZI proton assignment.

3.10. Mass spectrometry [34]

The mass spectrum of azithromycin was obtained utilizing A Thermo Scientific TSQ Quantum mass spectrometer systems feature HyperQuad quadrupoles. The MS analysis was performed with electro-spray ionization (ESI) interface in both negative and positive ion modes.

TSQ Quantum Ultra AM Instrument Conditions: Ionization mode and source: Positive and negative ESI; electrospray voltage: (+) 3.5 kV, (–) –2.5 kV; sheath gas: 1; auxiliary gas: 0; ion transfer tube temperature: 270 °C; ion transfer tube offset: 35 V; tube lens offset: 77 V; collision energy: 25 eV (famotidine), 23 eV (azithromycin); collision pressure: 1.2 mTorr; Q1/Q3 resolution: 0.1 Da FWHM; accurate mass mode: internal; micro scans: 2.

Figure 1.12 shows the detected mass fragmentation pattern of azithromycin. The major peaks in the spectrum occur at m/z 749, 591, 574, 434, 158.

For protonated azithromycin, elimination of H₂O and successive loss of the two sugar moieties were the major fragmentation pathways. The CID mass spectrum of the $[M - H]^-$ of azithromycin was very similar to that of the protonated species and showed successive loss of the two sugar moieties as the major dissociation pathways as shown in Figure 1.13.



4. METHODS OF ANALYSIS

4.1. Compendial methods of analysis

4.1.1 Identification

4.1.1.1 IR spectrum of Azithromycin

The IR spectra of the drug were obtained in the solid state using 90 g/l solutions in methylene chloride [21,34]

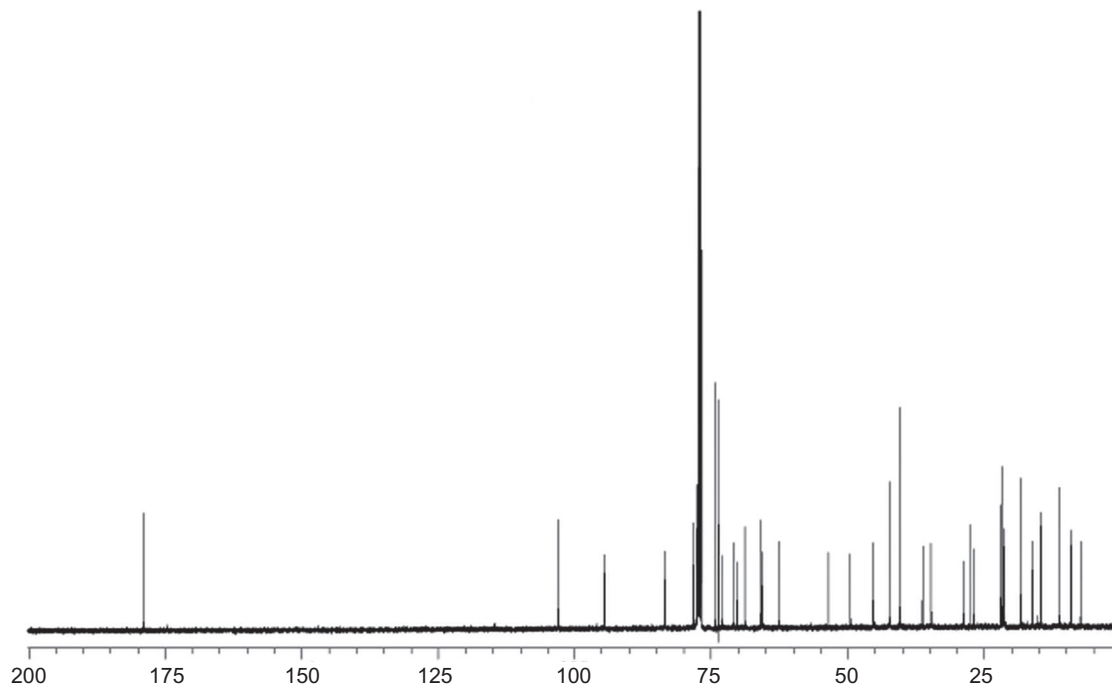


Figure 1.11 Full ^{13}C NMR spectrum of azithromycin in CDCl_3 .

Table 1.3 Assignments for the resonance bands observed in the ^{13}C NMR spectrum of azithromycin

Chemical shift (ppm)	Assignments	Chemical shift (ppm)	Assignment
178.9	(<u>C</u> ₁)	42.3	(2C, <u>C</u> ₄ and <u>C</u> ₇)
102.9	(<u>C</u> _{1'})	40.3	(<u>C</u> _{3'} —N(<u>C</u> ₃) ₂)
94.5	(<u>C</u> _{1''})	36.2	(<u>C</u> ₁)
83.3	(<u>C</u> ₅)	34.7	(<u>C</u> _{2''})
78.1	(<u>C</u> _{4''})	28.7	(<u>C</u> _{4'})
77.6	(<u>C</u> ₃)	27.6	(<u>C</u> ₆ — <u>C</u> ₃)
77.4	(<u>C</u> ₁₃)	26.7	(<u>C</u> ₈)
73.6	(<u>C</u> ₆)	22.0	(<u>C</u> ₈ — <u>C</u> ₃)
73.6	(<u>C</u> ₁₁)	21.6	(<u>C</u> _{3''} — <u>C</u> ₃)
72.9	(<u>C</u> _{3''})	21.3	(<u>C</u> _{5'} — <u>C</u> ₃)
70.8	(<u>C</u> _{2'})	21.3	(<u>C</u> ₁₃ — <u>C</u> ₂)
70.1	(<u>C</u> ₉)	18.2	(<u>C</u> _{5''} — <u>C</u> ₃)
68.7	(<u>C</u> _{5'})	16.2	(<u>C</u> ₁₂ — <u>C</u> ₃)
65.9	(<u>C</u> _{3'})	14.6	(<u>C</u> ₂ — <u>C</u> ₃)
65.5	(<u>C</u> _{5''})	11.2	(<u>C</u> ₁₃ —CH ₂ — <u>C</u> ₃)
62.4	(<u>C</u> ₁₀)	9.0	(<u>C</u> ₄ — <u>C</u> ₃)
49.4	(<u>C</u> _{3''} —O <u>C</u> ₃)	7.3	(<u>C</u> ₁₀ — <u>C</u> ₃)
45.3	(<u>C</u> ₂)		

4.1.1.2 HPLC drug chromatogram

The principal peak in the HPLC drug chromatogram obtained with test solution was similar in retention time and size to the principal peak in the chromatogram obtained with reference solution [21,34].

4.1.2 Impurity Analysis [47]

The specified impurities (A, B, C, D, E, F, G, H, I, K, J, L, M, N, O, and P) of azithromycin were determined using liquid.

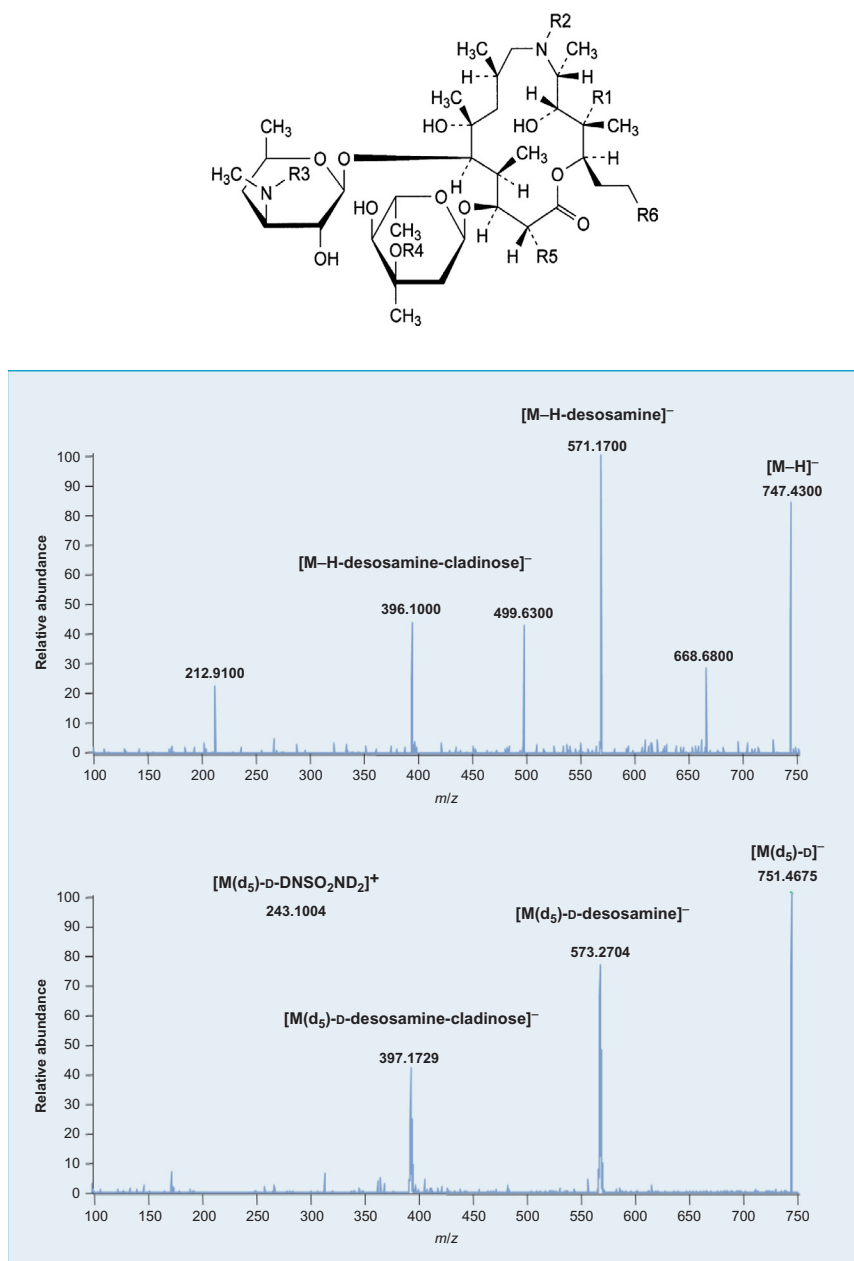


Figure 1.12 Negative ion ESI mass spectra of azithromycin (MW = 748): CID product ion spectra (MS/MS) of $[M - H]^-$ at m/z 747 and the fully exchanged $[M(d_5)-D]^-$ at m/z 751. Deuteration was achieved by liquid phase H/D exchange method. MS and MS/MS experiments were performed on a TSQ Quantum Ultra AM mass spectrometer.

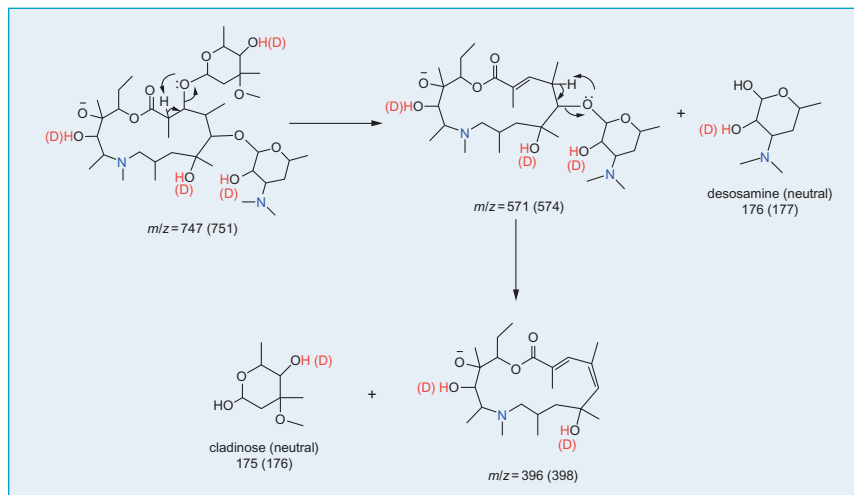
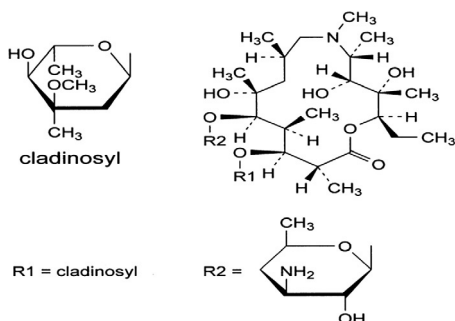
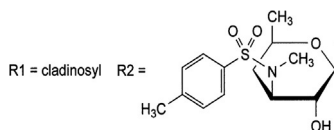


Figure 1.13 Proposed CID fragmentation mechanisms for the major fragment ions from deprotonated azithromycin at m/z 747 determined from H/D exchange patterns, high-resolution mass measurements, and MS/MS experiments. Numbers in parentheses refer to deuterated fragmentations. The proposed site of deprotonation is based on the most acidic proton of the lactone ring.

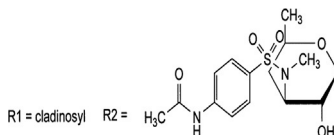
- (A) $R_1 = \text{OH}$, $R_2 = R_6 = \text{H}$, $R_3 = R_4 = R_5 = \text{CH}_3$: 6-demethylazithromycin,
 (B) $R_1 = R_6 = \text{H}$, $R_2 = R_3 = R_4 = R_5 = \text{CH}_3$: 3-deoxyazithromycin (azithromycin B),
 (C) $R_1 = \text{OH}$, $R_2 = R_3 = R_5 = \text{CH}_3$, $R_4 = R_6 = \text{H}$: 3''-O-demethylazithromycin (azithromycin C),
 (D) $R_1 = \text{OH}$, $R_2 = R_3 = R_4 = \text{CH}_3$, $R_5 = \text{CH}_2\text{OH}$, $R_6 = \text{H}$: 14-demethyl-14-(hydroxymethyl)azithromycin (azithromycin F),
 (F) $R_1 = \text{OH}$, $R_2 = R_4 = R_5 = \text{CH}_3$, $R_3 = \text{CHO}$, $R_6 = \text{H}$: 3'-N-demethyl-3'-N-formylazithromycin,
 (I) $R_1 = \text{OH}$, $R_2 = R_4 = R_5 = \text{CH}_3$, $R_3 = R_6 = \text{H}$: 3'-N-demethylazithromycin,
 (O) $R_1 = \text{OH}$, $R_2 = R_3 = R_4 = R_5 = R_6 = \text{CH}_3$: 2-desethyl-2-propylazithromycin,



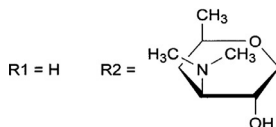
(E) 3'-(*N,N*-didemethyl)azithromycin (aminoazithromycin),



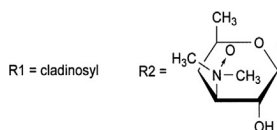
(G) 3'-*N*-demethyl-3'-*N*-[(4-methylphenyl)sulphonyl]azithromycin,



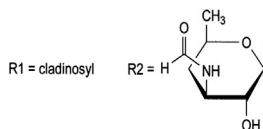
(H) 3'-*N*-[[4-(acetylamino)phenyl]sulphonyl]-3'-*N*-demethylazithromycin,



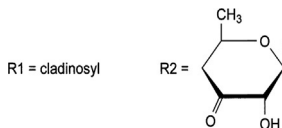
(J) 13-*O*-decladinosylazithromycin,



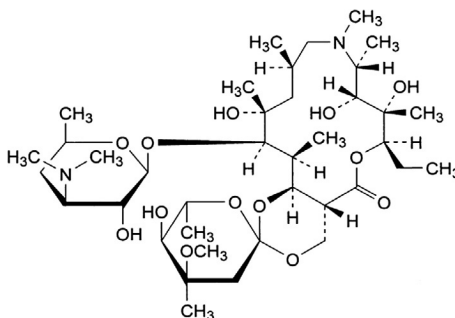
(L) azithromycin 3'-N-oxide,



(M) 3'-(N,N-didemethyl)-3'-N-formylazithromycin



(N) 3'-de(dimethylamino)-3'-oxoazithromycin,



(K) C₁₄, 1''-epoxyazithromycin (azithromycin E),
Unknown structure.

4.1.3 Other tests

4.1.3.1 Water [47]

1.8–6.5%, determined on 0.200 g.

4.1.3.2 Sulphated ash [47]

Maximum 0.2%, determined on 1.0 g.

4.1.4 Assay method [47]

4.1.4.1 Liquid chromatography

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, $\varnothing=4.6$ mm; stationary phase: octadecylsilyl vinyl polymer for chromatography R ($5\text{ }\mu\text{m}$); temperature: $40\text{ }^{\circ}\text{C}$.
- *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; UV spectrophotometry detection at λ 210 nm; injection, 10 μl ; run time, 15 min; retention time of Azithromycin, 10 min; resolution: minimum 3.0 between the peaks of impurity A and azithromycin.

4.2. Electrochemical methods of analysis

4.2.1 Voltammetry

Studies on the electrochemical oxidation and determination of azithromycin on glassy carbon and modified glassy carbon electrodes have been frequently published. The voltammetric determination of azithromycin at a carbon paste electrode [48], the adsorptive stripping voltammetric determination of azithromycin at a glassy carbon electrode modified by electrochemical oxidation [49], and the voltammetric assay of azithromycin in pharmaceutical dosage forms [50] have been published. Also, studies on the electrochemical oxidation of azithromycin and its interaction with bovine serum albumin [51], identification of azithromycin by abrasive stripping voltammetry [52], and the mathematical modeling of the electrode process of azithromycin using cyclic voltammetry at a hanging mercury drop electrode [53] can be found in the literature. There is also some data concerning a validated LC method for *in vitro* analysis of azithromycin using

electrochemical detection with dual glassy carbon electrodes operating in the oxidative screen mode [37]. The electro-chemical oxidation of azithromycin using voltammetry and *in situ* FTIR spectroscopy to obtain mechanistic information about the overall process on a platinum electrode in acetonitrile has also been reported [54].

Avramov *et al.* [55] examined the oxidative properties and assay of azithromycin at a gold electrode in neutral electrolyte using cyclic linear sweep voltammetry. The maximum value of the current of the oxidation peak of pure azithromycin and azithromycin from tablet dosage form (Hemomycin[®], Hemofarm, Vrsac, Serbia and Montenegro) at 0.6 V versus SCE in 0.05 M NaHCO₃ and in a mixture methanol–0.05 M NaHCO₃ (1:1) at a scan rate of 50 mV s⁻¹ is a linear function of the concentration in the range of 0.235–0.588 mg/cm³. HPLC analysis of the bulk of electrolyte confirmed the data obtained by analysis of the values of the current peak concerning the concentration of antibiotic in the investigated concentration range.

For the determination of the azithromycin concentration, only the first cycle was recorded, which will be presented later for different concentrations. It is obvious from the cyclic voltammograms of pure azithromycin in 0.05 M NaHCO₃ (Figures 1.14)

4.2.2 Coulometry

A variant of the Karl Fischer water determination was described [25]. By heating the drug substance, the contained water was transferred into a titration cell by a carrier gas. The automated system consisted of an oven sample processor and a coulometer.

4.3. Spectroscopic methods of analysis

4.3.1 Spectrophotometry

Azithromycin was determined in its pharmaceutical dosage forms by the formation of an ion pair between this drug and an inorganic complex of (Mo (V)–thiocyanate) followed by its extraction with dichloroethane. This ion-association complex shows an orange color and exhibits a maximum absorbance at 469 nm [56].

Su-Ying [57] established a UV spectrophotometry method to determine the contents of azithromycin tablets quickly and exactly. The concentration of azithromycin was determined according to related drug dissolubility standard procedures in pharmacopoeia. Ethanol and 0.1 mol/L hydrochloric

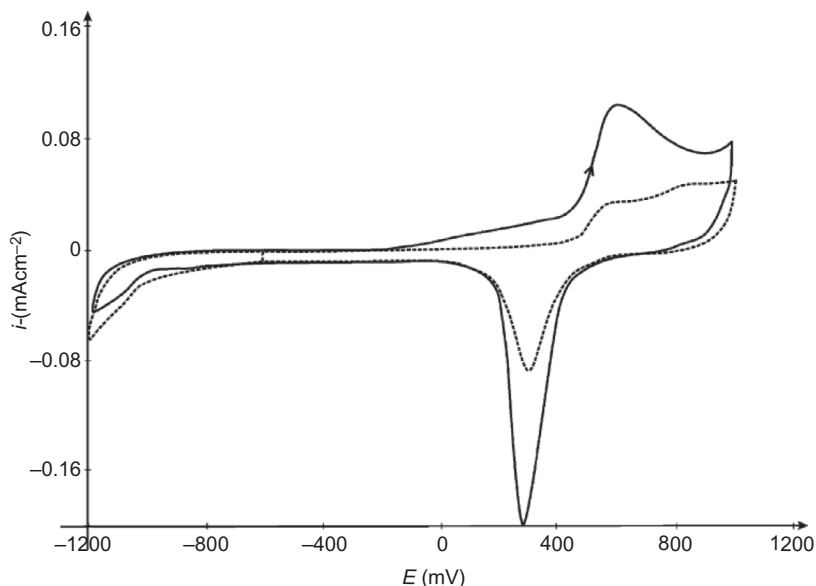


Figure 1.14 Cyclic voltammogram of an Au electrode in 0.05 M NaHCO_3 (---) and after the addition of 0.588 mg/cm^3 of pure azithromycin dihydrate, 30th sweep (full line), sweep rate: 50 mV/s.

acid were used as dilute solvents and 75 $\rightarrow$ 100 sulfuric acid was used as colored solvent. The samples were analyzed at the wavelength of 482 nm.

Huakan *et al.* [58] developed a spectrophotometric method for the determination of azithromycin based on the charge transfer reaction between azithromycin as donor and alizarin as acceptor in ethanol solution. The composition ratio and stability constant of charge transfer complex were 1:1 and 4.8×10^3 , respectively. The apparent molar absorptivity of complex at 546 nm is $5.79 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$.

Suhagia *et al.* [59] developed a simple and sensitive spectrophotometric method for the determination of azithromycin in its pharmaceutical dosage forms. In the method, azithromycin is oxidized with potassium permanganate to liberate formaldehyde, which is determined *in situ* using acetyl acetone, in the presence of ammonium acetate. A yellow colored chromogen was obtained, having an absorption maxima at 412 nm. The method is found to be linear in the concentration range of 10–75 $\mu\text{g/ml}$, with regression coefficient of 0.9978.

Shu-xia *et al.* [60] established a method for the determination of azithromycin tablets dissolution based on charge–transfer reaction with alizarin red. Methods of the dissolution test were conducted, using phosphate

buffer solution as medium, with a stirring speed of 100 rpm/min. The solution was withdrawn after 45 min. The absorbance of dissolution solutions was measured at 538 nm. Dissolution limit is 75% of the labeled amount. Result Good linear correlation was achieved at the range of 50–250 µg/ml azithromycin ($r=0.9996$).

Paula *et al.* [61] proposed a new method for simple and fast spectrophotometric determination of azithromycin in pharmaceutical formulations. The method is based on the charge transfer reaction between the azithromycin and quinalizarin in methanol medium. In order to achieve maximum sensitivity, the effect of some chemical variables such as the type of solvent, reagent concentration, and reaction time was evaluated. The reaction was characterized in terms of stability of the product formed and its stoichiometry, and the apparent molar absorptivity and association constant were derived. Best conditions for the analytical determination of azithromycin were observed in methanol medium with a quinalizarin concentration of 50 mg L⁻¹. At these conditions, the radical anion (absorbing species) was formed in the medium immediately after mixing of the reagents and showed maximum absorption at 564 nm. The method presented a limit of detection of 0.35 mg L⁻¹ and a limit of quantification of 1.2 mg L⁻¹.

Sayed *et al.* [62] developed two simple, accurate, precise, and rapid spectrophotometric and conductometric methods for the estimation of erythromycin thiocyanate(I), clarithromycin (II), and azithromycin dihydrate(III) in both pure and pharmaceutical dosage forms. The spectrophotometric procedure depends on the reaction of rose Bengal and copper with the cited drugs to form stable ternary complexes which were extractable with methylene chloride, and the absorbances were measured at 558, 557, and 560 nm for (I), (II), and (III), respectively. The conductometric method depends on the formation of an ion-pair complex between the studied drug and rose Bengal.

Ashour *et al.* [63] developed and validated new, simple, and rapid spectrophotometric for the assay of two macrolide drugs, azithromycin (AZT) and erythromycin (ERY), in pure and pharmaceutical formulations. The method was based on the reaction of AZT and ERY with sodium 1,2-naphthoquinone-4-sulphonate in alkaline medium at 25 °C to form an orange-colored product of maximum absorption peak at 452 nm.

4.3.2 Spectrofluorimetry

El-Rabbat *et al.* [64] described a simple spectrofluorometric method for the analysis of four macrolide antibiotics. The method is based on the condensation of 10% (w/v) malonic acid and acetic acid anhydride under

the catalytic effect of tertiary amine groups of the studied macrolides. The relative fluorescence intensity of the condensation product was measured at 397/452 nm (excitation/emission) for azithromycin dihydrate and at 392/445 nm (for clarithromycin, erythromycin ethylsuccinate, and roxithromycin).

Almeida *et al.* [65] proposed a fluorescence method for azithromycin determination in pharmaceutical formulations. The method is based on the synchronous fluorescence ($\Delta\lambda = 30$ nm, 482 nm) produced when azithromycin is derivatized in strong acidic medium ($9.0 \text{ mol L}^{-1} \text{ HCl}$).

Khashaba [66] analyzed the macrolides (erythromycin, erythromycin esters, azithromycin dihydrate, clarithromycin, and roxithromycin) by a simple spectrofluorimetric method based on the oxidation by cerium (VI) in the presence of sulfuric acid and monitoring the fluorescence of cerium (III) formed at λ_{ex} 255 nm and λ_{em} 348 nm.

4.3.3 Colorimetry

Hunfeld *et al.* [67] used a newly developed colorimetric microdilution method to analyze the activity of 12 antimicrobial agents against nine *Borrelia burgdorferi* isolates, including all three genospecies pathogenic for humans. In addition, *in vitro* antimicrobial resistance patterns of *Borrelia valaisiana* and *Borrelia bissettii* tick isolates were investigated. The applied test system is based upon color changes that occur in the presence of phenol red and result from the accumulation of nonvolatile acid produced by actively metabolizing spirochetes. After 72 h of incubation, minimal inhibitory concentrations (MICs) were determined from the decrease of absorbance by software-assisted calculation of growth curves. MIC values were lowest for azlocillin (MIC, $\leq 0.125 \text{ }\mu\text{g/ml}$), ceftriaxone (MIC range, $\leq 0.015\text{--}0.06 \text{ }\mu\text{g/ml}$), and azithromycin (MIC range, $\leq 0.015\text{--}0.06 \text{ }\mu\text{g/ml}$). Whereas tobramycin (MIC range, $8\text{--}64 \text{ }\mu\text{g/ml}$) exhibited little activity, spectinomycin (MIC range, $0.25\text{--}2 \text{ }\mu\text{g/ml}$) showed *in vitro* antimicrobial activity against *B. burgdorferi*.

Haleem *et al.* [68] developed a simple, accurate, and rapid spectrophotometric method for the estimation of azithromycin by the acidic hydrolysis of the drug with sulfuric acid and monitoring the absorbance at 482 nm.

4.4. Chromatographic methods of analysis

4.4.1 Electrophoresis

Kumar *et al.* [69] described the use of AZM as a chiral selector for the enantiomeric separations of five chiral drugs and one amino acid (tryptophan) in

capillary electrophoresis (CE). The enantioseparation is carried out using polar organic mixtures of acetonitrile (ACN), methanol (MeOH), acetic acid, and triethylamine as run buffer. The influences of the chiral selector concentration, ACN/MeOH ratio, applied voltage, and capillary temperature on enantioseparation are investigated. The results shown that AZM is a viable chiral selector in CE for the enantioseparation of the type of chiral drugs investigated.

Lebedeva *et al.* [70] described successful use of macrolide antibiotic azithromycin for enantioseparation of tetrahydrozoline. The procedure was proposed for the analysis of tetrahydrozoline in pharmaceuticals. Linearity was achieved in the concentration range 5×10^{-2} to 1 mg/ml. The azithromycin stability in the background electrolyte and the antibiotic adsorption on the fused-silica capillary were studied. Best enantioseparation with resolution factor 1.6 was achieved in less than 10 min.

4.4.2 Thin-layer chromatography

Kwiecień *et al.* [71] established a thin-layer chromatographic (TLC) method with densitometric detection for quantification of azithromycin in pharmaceutical preparations. Silica gel plates with fluorescence indicator F₂₅₄ were used with chloroform–ethanol–ammonia 6:14:0.2 (v/v) as mobile phase. Chromatograms were visualized by spraying with 1:4 (v/v) sulfuric acid–ethanol and heating at 120 °C for 5 min. Scanning and densitometric analysis was performed at 483 nm. The R_F of azithromycin under these conditions was 0.53. The method was characterized by high sensitivity (LOD = 40 ng/zone and LOQ = 80 ng/zone), wide linear range (from 0.08 to 1.2 µg/zone, $r = 0.9965$)

Khedr *et al.* [72] described a validated stability-indicating TLC method of the analysis of azithromycin (AZT) in bulk and capsule forms. Both AZT potential impurity and degradation products can be selectively and accurately estimated in both raw material and product onto one precoated silica–gel TLC plate 60F₂₅₄. The development system used is *n*-hexane–ethyl acetate diethylamine (75:25:10, v/v/v). The separated bands are detected as brown to brownish red spots after spraying with modified Dragendorff's solution. The R_F values of AZT, azaerythromycin A, and the three degradation products are 0.54, 0.35, 0.40, 0.20, and 0.12, respectively.

4.4.3 High-performance liquid chromatography

Liquid chromatography with UV detection has been already employed for the analysis of azithromycin in azithromycin tablets [39], raw material, and

azithromycin tablets [36,73]. A high-performance liquid chromatography (HPLC) method with UV detector was developed for the determination of azithromycin and other related compounds, impurities, degradation products in raw material as well as in new pharmaceutical formulations: dry suspension and capsules. Validation of the method was performed according to the requirements of USP for assay determination, which included accuracy, precision, specificity, linearity, and range.

An HPLC method was developed that confirmed the photodegradation of azithromycin in environmental waters, under simulated solar radiation [74].

A stability-indicating HPLC method has been described for azithromycin in raw materials, capsules, and suspension [75].

Other HPLC methods for the analysis of AZI in raw material, dosage forms, and biological samples that have been reported in the literature are listed in Table 1.4.

4.5. Determination in body fluids and tissues

Azithromycin concentrations versus time profiles in extracellular space of muscle and subcutaneous adipose tissue, also in plasma and white blood cells, were determined at days 1 and 3 of treatment as well as 2 and 7 days after end of treatment. Of all compartments, azithromycin concentrations were highest in white blood cells, attesting for intracellular accumulation. However, azithromycin concentrations in both soft tissues were markedly lower than in plasma both during and after treatment. Azithromycin concentrations were measured by subinhibitory at all time points in both soft tissues and at the large majority of observed time points in plasma [76].



5. STABILITY

El-Gindy *et al.* [75] developed a validated stability-indicating HPLC method for the analysis of azithromycin (AZ) and its related compounds in raw materials and capsules. The stability of AZ was studied under accelerated acidic, alkaline, and oxidative conditions. The major peak detected from the degradation of AZ in alkaline and acidic conditions was decladinosylazithromycine, while azithromycin N-oxide was detected from the oxidative degradation. Long-term stability studies for capsule and oral suspension were also carried out.

Table 1.4 HPLC methods for the analysis of azithromycin

Column	Sample matrix	Mobile phase composition	Detection	References
Gamma-alumina	Raw material	Phosphate buffer–acetonitrile, adjusted to pH 11.0 with potassium hydroxide	Amperometric guard: +0.70 V screen: +0.85 V	[35]
LiChroCART [®] C ₁₈ , 5 µm	Raw material	Phosphate buffer–acetonitrile–methanol, adjusted to pH 8.0 with phosphoric acid	UV 215 nm	[36]
Nova-Pack C ₁₈ , 4 µm	Raw material	Ammonium acetate–acetonitrile–methanol tetrahydrofuran, mobile phase pH 7.2–7.4	Amperometric guard: +0.7 V screen: +0.8 V	[37]
XTerra RP C ₁₈ , 5 µm	Raw material	Phosphate buffer–water–acetonitrile, adjusted to pH 6.5 with potassium hydroxide	UV 215 nm	[38]
Phenomenex Synergi [®] C ₁₈ , 4 µm	Raw material, dosage forms	Gradient elution, phosphate buffer–acetonitrile–methanol, adjusted to pH 7.0 with potassium hydroxide	UV 210 nm	[39]
YMC-Park ODS-AP C ₁₈ , 5 µm	Rat's plasma	Phosphate buffer–acetonitrile, adjusted to pH 7.2 with potassium hydroxide	Amperometric detect: +0.95 V	[40]

Continued

Table 1.4 HPLC methods for the analysis of azithromycin—cont'd

Column	Sample matrix	Mobile phase composition	Detection	References
Nova-Pack C ₁₈ , 4 µm	Human tears and plasma	Phosphate buffer–sodium perchlorate–acetonitrile–methanol, adjusted to pH 7.0 with phosphoric acid	Amperometric guard: +0.7 V screen: +0.85 V	[41]
Radial-Pak Resolve Silica cartridge, 5 µm	Rat's blood plasma, serum, and human urine	Ammonium acetate–acetonitrile–methanol, adjusted to pH 7.0 with acetic acid	Coulometric guard: +0.90 V	[42]
C ₁₈ (250 mm × 4.6 mm, 5 µm)	Azithromycin syrup	Acetonitrile –0.067 mol L ^{–1} K ₂ HPO ₃ (pH adjusted to 6.5) (40:60)	UV 210 nm	[43]
ODS–C ₁₈ column (150 mm × 4.6 mm, 5 µm)	Eye drops	Acetonitrile and 0.1 mol L ^{–1} KH ₂ PO ₄ as the mobile phase (30:70)	UV 215 nm	[44]
CAPCELL PAK C ₍₁₈₎ MGIIcolumn (250 mm × 4.6 mm, 5 µm)	Azithromycin capsules	Acetonitrile phosphate buffer (to dissolve dipotassium hydrogen phosphate 8.7 g diluting to 1000 ml with water, and adjust pH to 8.2 with phosphoric acid) (60:40)	UV 210 nm	[45]
Dikma Technologies Diamonsil C ₁₈ column (150 mm × 4.6 mm, 5 µm)		Ammonium dihydrogen phosphate (0.045 M, pH 3.0 adjusted by phosphoric acid):acetonitrile 47:15 (v/v)	UV 210 nm	[46]

Moreno *et al.* [77] were developed a stability study of azithromycin in ophthalmic preparations by submission to different types of light, temperature, and pH, using the biodiffusion assay (cylinder 3 × 3) for the quantifications. *Bacillus subtilis*, ATCC 9372, was used as test organism. The used concentration range was of 50–200 µg/ml.



6. CLINICAL APPLICATIONS

6.1. An overview

Azithromycin is the member of macrolide antibiotics. It is semisynthetic derivatives of erythromycin. Azithromycin differs from erythromycin by the addition of a methyl-substituted nitrogen atom into the lactone ring. This structural modification improved acid stability and tissue penetration and broaden the spectrum of activity. Macrolides generally cover a wide range of Gram-positive and Gram-negative bacterial species including intracellular pathogens such as *Chlamydia* and *Legionella*. They express their antibiotic activity by binding to the 50S ribosome subunit and inhibit protein synthesis [78,79].

Azithromycin pharmacology and therapeutics aspects were given below in more details.

6.2. Antimicrobial spectrum susceptibility

Azithromycin is less active against Gram-positive bacteria than erythromycin but is considerably more effective against some Gram-negative organisms such as *Haemophilus influenzae*, *Moraxella catarrhalis* as well as having activity against some of the Enterobacteriaceae such as *Escherichia coli* and *Salmonella* and *Shigella* species; Also activity against *Legionella pneumophila*, *B. burgdorferi*, *Mycoplasma pneumoniae*. Also it is owes enhanced activity against *Mycobacterium avium-intracellulare*, as well as against some protozoa (e.g., *Cryptosporidium*, and *Plasmodium* species, an excellent action against *Toxoplasma gondii*, killing the cysts). Azithromycin more active than erythromycin against *Chlamydia trachomatis* and *Ureaplasma urealyticum*, [79,80].

6.3. Mechanism of action

It is a bacteriostatic agent that inhibits protein synthesis by binding reversibly to 50S ribosomal subunits of sensitive microorganisms. Cells are considerably more permeable to the unionized form of the drug, which probably explains the increased antimicrobial activity at alkaline pH [79].

6.4. Resistance to macrolides

Generally, resistance to macrolides results from the following mechanisms:

- Drug efflux by an active pump mechanism
- Ribosomal protection by inducible or constitutive production of methylesterase enzymes, mediated by expression of (ermA), (ermB), and (ermC), which modify the ribosomal target and decrease drug binding
- Hydrolysis by esterases produced by Enterobacteriaceae
- Chromosomal mutations that alter a 50S ribosomal subunit protein (found in *B. subtilis*, *Campylobacter* species, mycobacteria, and Gram-positive cocci) [79].

6.5. Actions other than antimicrobial effects

- Studies were indicated the activity of azithromycin as promising medication for the treatment of gastroparesis and gastrointestinal dysmotility [81,82].
- One study showed that azithromycin may be effective against late-onset asthma [83].
- Azithromycin has been shown to be effective against malaria when used in combination with artesunate or quinine. Azithromycin–artesunate, even when given only once daily for 3 days, and azithromycin–quinine, given 3 times daily, are safe and efficacious combination treatments for uncomplicated falciparum malaria, and they deserve additional study in special patient populations [84].
- *Ischaemic heart disease*: Macrolide antibacterials, including azithromycin, clarithromycin, and roxithromycin, have been investigated in the prevention of ischaemic heart disease, based on a suggested link between atherosclerosis and infection with *Chlamydia pneumoniae* (*Chlamydia pneumoniae*). Although preliminary results from some pilot studies were promising, longer-term studies in large numbers of patients were disappointing and none of the three macrolides decreased ischemic events or provided clinical benefit; indeed, in one study, an unexpected increase in cardiovascular mortality was seen in those taking clarithromycin [79,86,87].
- *Gingival hyperplasia*: Azithromycin improves cyclosporin-associated gingival hyperplasia, especially when administered early in the process [88].
- *Cystic fibrosis*: Long-term azithromycin is widely used in cystic fibrosis, with evidence demonstrating a reduction in lung function decline and exacerbation rate. This immunomodulatory therapy probably disrupts *Pseudomonas aeruginosa* biofilm growth [89].

6.6. Clinical uses and dosing

Clinically, azithromycin is used for many as follows: respiratory-tract infections, otitis media, skin and soft-tissue infections, uncomplicated genital chlamydial infections and nongonococcal urethritis, mild or moderate typhoid due to multiple-antibacterial-resistant organisms, prophylaxis of group A streptococcal infection and as prevention therapy of bacterial endocarditis in patients undergoing dental procedures who are at high risk for endocarditis, pertussis, mycobacterial infections [79,88,90].

- Treatment or prophylaxis of *Mycobacterium avium-intracellulare* infection in AIDS patients requires higher doses: 600 mg daily in combination with one or more other agents for treatment, or 1200 mg once weekly for primary prevention.
- Azithromycin is useful in the treatment of sexually transmitted diseases, especially during pregnancy when tetracyclines are contraindicated. The treatment of uncomplicated nongonococcal urethritis presumed to be caused by *C. trachomatis* consists of a single 1-g dose of azithromycin. This dose also is effective for chancroid [79].
- Azithromycin (1 g per week for 3 weeks) is an alternative regimen for the treatment of *granuloma inguinale* or *lymphogranuloma venereum* [79].
- Uncomplicated genital chlamydial infections and nongonococcal urethritis, 1 g as a single dose [90].
- Typhoid, 500 mg once daily for 7 days [90].

6.7. ADME profile

Azithromycin administered orally is absorbed rapidly and distributes widely throughout the body, except to the brain and cerebrospinal fluid. Peak plasma concentrations occur 2–3 h after an oral dose. A 500-mg loading dose will produce a peak plasma drug concentration of ~0.4 g/ml. When this loading dose is followed by 250 mg once daily for 4 days, the steady-state peak drug concentration is 0.24 g/ml. Azithromycin also can be administered intravenously, producing plasma concentrations of 3–4 g/ml after a 1-h infusion of 500 mg. Absorption from capsules, but not tablets or suspension, is reduced by food. Azithromycin's unique pharmacokinetic properties include extensive tissue distribution and high drug concentrations within cells (including phagocytes), resulting in much greater concentrations of drugs in tissue or secretions compared to simultaneous serum concentrations. Tissue fibroblasts act as the natural reservoir for the drug *in vivo*. Data from animal studies indicate that azithromycin crosses the placenta. Protein

binding is 50% at very low plasma concentrations and less at higher concentrations. Azithromycin undergoes some hepatic metabolism (demethylation) to inactive metabolites, but biliary excretion is the major route of elimination. Only 12% of drug is excreted unchanged in the urine. The elimination half-life ($t_{1/2}$), 40–68 h, is prolonged because of extensive tissue sequestration and binding [79,85].

6.8. Side effects

Anorexia, dyspepsia, flatulence, dizziness, headache, drowsiness, convulsions, arthralgia, and disturbances in taste and smell; rarely constipation, hepatitis, hepatic failure, syncope, insomnia, agitation, anxiety, asthenia, paraesthesia, hyperactivity, thrombocytopenia, haemolytic anaemia, interstitial nephritis, acute renal failure, photosensitivity, tooth and tongue discoloration [90].

6.9. Drug interactions

- Azithromycin, generally appear to be free of drug interactions. Caution is advised, nevertheless, when using azithromycin in conjunction with drugs known to interact with erythromycin [80].
- Giving azithromycin with antacids containing aluminum or magnesium salts can reduce the rate, but not the extent, of its absorption; azithromycin should be given at least 1 h before or 2 h after the antacid [85].
- Azithromycin serum concentrations are markedly increased when it is given with nelfinavir [85].
- Azithromycin capsules should not be administered with food because it will result in reduced absorption.
- Azithromycin possibly enhances anticoagulant effect of coumarins [90].
- Azithromycin possibly increases plasma concentration of theophylline [90].

REFERENCES

- [1] Z. Banić Tomišić, The story of azithromycin, *Kemija u Industriji* 60 (12) (2011) 603–617.
- [2] Martindale the Complete Drug Reference, 36th, the Pharmaceutical Press, 207.
- [3] <http://en.wikipedia.org/wiki/Azithromycin>.
- [4] <http://www.rxlist.com/zithromax-drug.htm>.
- [5] The Merck Index, 12th, 1996, Merck & CO., Inc., USA. 157.
- [6] http://www.who.int/selection_medicines/committees/expert/19/applications/azithromycin_21_ac_ad.pdf.
- [7] <http://www.drugbank.ca/drugs/db00207>.
- [8] Z.J. Li, A.V. Trask, Crystal forms of azithromycin, 2005, U.S. Patent No. 6,977,243.

- [9] S. Turchetta, P. Massardo, P. Casellato, Process for preparing high purity azithromycin, 2003, U.S. Patent Application 10/516,719.
- [10] S.P. Singh, S.M.J. Mukarram, M. Purohit, A.R. Khan, Process for preparation of anhydrous azithromycin, 2008, U.S. Patent Application 12/184,051.
- [11] M. Bayod-Jasanada, R.J. Carbajo, F. Lopez-Ortiz, Synthesis of 9-deoxy-9a-aza-9a-homoerythromycin A 11,12-hydrogen borate and azithromycin 11,12-hydrogen borate. A new procedure to obtain azithromycin dihydrate, *J. Org. Chem.* 62 (21) (1997) 7479.
- [12] S. Djokić, G. Kobrehel, G. Lazarevski, N. Lopotar, Z. Tamburašev, B. Kamenar, A. Nagl, I. Vicković, Erythromycin series. Part 11. Ring expansion of erythromycin A oxime by the Beckmann rearrangement, *J. Chem. Soc. Perkin Trans. 1* (1986) 1881–1890.
- [13] B.V. Yang, M. Goldsmith, J.P. Rizzi, A novel product from Beckmann rearrangement of erythromycin A 9 (E)-oxime, *Tetrahedron Lett.* 35 (19) (1994) 3025–3028.
- [14] J.A. De La Torre Garcia, F.F. Andrade, J.M.F.L. Ochoa, Single-step process for preparing 7, 16-deoxy-2-aza-10-0-cladinosil-12-0-desosaminil-4, 5-dihydroxy-6-ethyl-3, 5, 9, 11, 13, 15-hexamethylbicyclo (11.2.1) hexadeca-1 (2)-en-ona and obtaining a new form of 9-deoxy-9a-methyl-9a-aza-9a-homoerythromycin A, 2003, U.S. Patent 6,528,492.
- [15] S.H. Pine, B.L. Sanchez, Formic acid-formaldehyde methylation of amines, *J. Org. Chem.* 36 (6) (1971) 829–832.
- [16] J.S. Davies, E. Hunt, I.I. Zomaya, The chemistry of erythromycin. Reactions of erythromycin A imine and its 6-methyl ether with aldehydes and hydrazines, *J. Chem. Soc. Perkin Trans. 5* (1990) 1409–1414.
- [17] Y. Ni, Z. Sun, Recent progress on industrial fermentative production of acetone–butanol–ethanol by *Clostridium acetobutylicum* in China, *Appl. Microbiol. Biotechnol.* 83 (3) (2009) 415–423.
- [18] H.A. Kirst, Recent Progress in the Chemical Synthesis of Antibiotics, Springer, Heidelberg, 1990, pp. 39–63.
- [19] S. Mutak, Azalides from azithromycin to new azalide derivatives, *J. Antibiotics* 60 (2) (2007) 85–122.
- [20] S. Rengaraju, Process for the preparation of non-hygroscopic azithromycin dihydrate, 2002, U.S. Patent Application 10/050,897.
- [21] European Pharmacopoeia. ed. t. edition. vol. 16. 2005. 1649.
- [22] S. Babić, A.J.M. Horvat, D. Mutavdžić Pavlović, M. Kaštelan-Macan, Determination of pK_a values of active pharmaceutical ingredients, *TrAC Trends Anal. Chem.* 26 (11) (2007) 1043–1061.
- [23] Material Safety Data Sheet, 2012. <http://www.greenstonellc.com/pdfs/MSDS/AZITHROMYCIN%20TABLETS%20250MG%20AND%20500MG%20-%20MSDS.pdf>.
- [24] S. Odoemelam, E. Ogoko, B. Ita, N. Eddy, Inhibition of the corrosion of zinc in H_2SO_4 by 9-deoxy-9a-aza-9a-methyl-9a-homoerythromycin A (azithromycin), *Portugaliae Electrochim. Acta* 27 (1) (2009) 57–68.
- [25] R. Gandhi, O. Pillai, R. Thilagavathi, B. Gopalakrishnan, C.L. Kaul, R. Panchagnula, Characterization of azithromycin hydrates, *Eur. J. Pharm. Sci.* 16 (3) (2002) 175–184.
- [26] A.K. Tiwary, G.M. Panpalia, Influence of crystal habit on trimethoprim suspension formulation, *Pharm. Res.* 16 (2) (1999) 261–265.
- [27] Z.H. Zhang, T.S. Li, T.S. Jin, J.X. Wang, A simple and efficient procedure for deprotection of tetrahydropyranyl ethers catalysed by expansive graphite†, *J. Chem. Res. Synopses* 3 (1998) 152–153.
- [28] Z.J. Li, A.V. Trask, Crystal forms of azithromycin, 2006, Google Patents.
- [29] D.J.M. Allen, K.M. Nepveux, Azithromycin dihydrate, in *European Patent*, 1992, EP 0298650.

- [30] S. Timoumi, D. Mangin, R. Peczalski, F. Zagrouba, J. Andrieu, A.H. Abadi, Stability and thermophysical properties of azithromycin dihydrate, Arab. J. Chem. (2010). <http://www.sciencedirect.com/science/article/pii/S1878535210002297>.
- [31] <http://www.chemspider.com/chemical-structure.2278824.html>.
- [32] M.S.B. Jasanada, I.L. Garcia, F.F. Mari, Azithromycin preparation in its noncrystalline and crystalline dihydrate forms, 2002, U.S. Patent 6,451,990.
- [33] F.G. Glansdorp, R.J. Spandl, J.E. Swatton, O. Loiseleur, M. Welch, D.R. Spring, Using chemical probes to investigate the sub-inhibitory effects of azithromycin, Org. Biomol. Chem. 6 (22) (2008) 4120–4124.
- [34] A. Kamel, K. Colizza, G. Pgrd, C.T.P. Jeanville, T.F. Scientific, Mechanisms of Ion Formation for Famotidine and Azithromycin Using Hydrogen/Deuterium Exchange and High Resolution Mass Measurements, Application Note: 374.
- [35] L. Tong, P. Eichhorn, S. Pérez, Y. Wang, D. Barceló, Photodegradation of azithromycin in various aqueous systems under simulated and natural solar radiation: Kinetics and identification of photoproducts, Chemosphere 83 (3) (2011) 340–348.
- [36] A. Khedr, M. Sheha, Quantitative thin-layer chromatographic method of analysis of azithromycin in pure and capsule forms, J. Chromatogr. Sci. 41 (1) (2003) 10–16.
- [37] R.A. Shawabkeh, M.F. Tutunji, Mathematical modelling of the electrode process of azithromycin using cyclic voltammetry at hanging mercury drop electrode, Sensors 2 (11) (2002) 436–446.
- [38] A. El-Gindy, K.A. Attia, M.W. Nassar, N.M. Al Abasawi, M. Al-Shabrawi, Optimization and validation of a stability-indicating RP-HPLC method for determination of azithromycin and its related compounds, J. AOAC Int. 94 (2) (2011) 513–522.
- [39] A. Kwiecień, J. Krzek, Ł. Biniak, TLC–densitometric determination of azithromycin in pharmaceutical preparations, JPC 21 (3) (2008) 177–181.
- [40] U. Pharmacopeia. 32, 2009, in US Pharmacopeial Convention, Rockville, MD.
- [41] F. Kamau, H. Chepkwony, J. Ngugi, D. Debremaeker, E. Roets, J. Hoogmartens, Isocratic liquid chromatographic method for the analysis of azithromycin and its structurally related substances in bulk samples, J. Chromatogr. Sci. 40 (9) (2002) 529–533.
- [42] C. Taninaka, H. Ohtani, E. Hanada, H. Kotaki, H. Sato, T. Iga, Determination of erythromycin, clarithromycin, roxithromycin, and azithromycin in plasma by high-performance liquid chromatography with amperometric detection, J. Chromatogr. B Biomed. Sci. Appl. 738 (2) (2000) 405–411.
- [43] D.A. Raines, A. Yusuf, M.H. Jabak, W.S. Ahmed, Z.A. Karciglu, A. El-Yazigi, Simultaneous high-performance liquid chromatography analysis of azithromycin and two of its metabolites in human tears and plasma, Ther. Drug Monit. 20 (6) (1998) 680–684.
- [44] L. Ke, Z. Yajie, Determination of azithromycin and azithromycin capsules by HPLC [J], China Pharm. 1 (2010) 041.
- [45] Y.H. Kim, J.V. Pothuluri, C.E. Cerniglia, Voltammetric investigation of macrolides by an HPLC–coulometric assay, J. Pharm. Biomed. Anal. 38 (3) (2005) 390–396.
- [46] H. Yan, H. Yuan, Determination of azithromycin in azithromycin syrup by HPLC [J], Anhui Med. Pharm. J. 1 (2009) 014.
- [47] British Pharmacopoeia. vol. 1. 2005, the stationery office on behalf of the medicine and health care products Regulatory Agency (MHRA), pp. 192–194.
- [48] M.A.R. Gaur, J. Gan, P. Hansal, K. Harper, R. Mannan, A. Panchal, K. Patel, M. Patel, N. Patel, J. Rana, A. Rogowska, British Pharm. (2009) 469–477.
- [49] O.A. Farghaly, N.A. Mohamed, Voltammetric determination of azithromycin at the carbon paste electrode, Talanta 62 (3) (2004) 531–538.
- [50] B. Nigovic, Adsorptive stripping voltammetric determination of azithromycin at a glassy carbon electrode modified by electrochemical oxidation, Anal. Sci. 20 (4) (2004) 639–643.
- [51] B. Nigović, B. Šimunić, Voltammetric assay of azithromycin in pharmaceutical dosage forms, J. Pharm. Biomed. Anal. 32 (1) (2003) 197–202.

- [52] W. Yunhua, J. Xiaobo, H. Shengshui, Studies on electrochemical oxidation of azithromycin and its interaction with bovine serum albumin, *Bioelectrochemistry* 64 (1) (2004) 91–97.
- [53] Š. Komorsky-Lovrić, B. Nigović, Identification of 5-aminosalicylic acid, ciprofloxacin and azithromycin by abrasive stripping voltammetry, *J. Pharm. Biomed. Anal.* 36 (1) (2004) 81–89.
- [54] R. Gandhi, C. Kaul, R. Panchagnula, Validated LC method for in-vitro analysis of azithromycin using electrochemical detection, *J. Pharm. Biomed. Anal.* 23 (6) (2000) 1073.
- [55] J.A.R.L. Ortiz, O.P. Marquez, J. Marquez, In: Proceedings of the 2001 Joint International Meeting—The 200th Meeting of the Electrochemical Society Inc. and the 52nd Annual Meeting of the International Society of Electrochemistry, San Francisco, CA, 2001, pp. 2–7(Abs# 1192).
- [56] M. Avramov Ivić, S. Petrović, D.Ž. Mijin, P. Živković, I. Kosović, K. Drljević, M. Jovanović, Studies on electrochemical oxidation of azithromycin and Hemomycin® at gold electrode in neutral electrolyte, *Electrochim. Acta* 51 (12) (2006) 2407–2416.
- [57] M. Rachidi, J. Elharti, K. Digua, Y. Cherrah, A. Bouklouze, New spectrophotometric method for azithromycin determination, *Anal. Lett.* 39 (9) (2006) 1917–1926.
- [58] M. Su-Ying, Determination of the contents of azithromycin tablets with UV spectrophotometry [J], *J. Pediatr. Pharm.* 4 (2007) 020.
- [59] L. Huakan, Z. Yanqing, W. Yuhua, K. Janfeng, Spectrophotometric determination of azithromycin based on the charge transfer reaction between azithromycin and alizarin, *Chin. J. Anal. Chem.* 32 (5) (2004) 598–600.
- [60] B. Suhagia, S. Shah, I. Rathod, H. Patel, K. Doshi, Determination of azithromycin in pharmaceutical dosage forms by spectrophotometric method, *Indian J. Pharm. Sci.* 68 (2) (2006) 242.
- [61] T. Shu-Xia, J. Ye, X. Zan, C. Tai-Mei, Z. Xing-Ru, Spectrophotometric determination of azithromycin tablets dissolution by charge-transfer complex with alizarin red, *Chin. J. Antibiot.* 30 (9) (2005) 533.
- [62] C.E.R. De Paula, V.G.K. Almeida, R.J. Cassella, Novel spectrophotometric method for the determination of azithromycin in pharmaceutical formulations based on its charge transfer reaction with quinalizarin, *J. Braz. Chem. Soc.* 21 (9) (2010) 1664–1671.
- [63] R.A. Sayed, W.S. Hassan, M.Y. El-Mammli, A. Shalaby, New spectrophotometric and conductometric methods for macrolide antibiotics determination in pure and pharmaceutical dosage forms using rose Bengal, *J. Spectrosc.* 7 (2013) 13–26, V 2013, ID2 14270, p.13.
- [64] <http://dx.doi.org/10.1155/2013/214270>.
- [65] S. Ashour, R. Bayram, Novel spectrophotometric method for determination of some macrolide antibiotics in pharmaceutical formulations using 1,2-naphthoquinone-4-sulphonate, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 99 (2012) 74–80.
- [66] N. El-Rabbat, H.F. Askal, P.Y. Khashaba, N.N. Attia, A validated spectrofluorometric assay for the determination of certain macrolide antibiotics in pharmaceutical formulations and spiked biological fluids, *J. AOAC Int.* 89 (5) (2006) 1276–1287.
- [67] V.G. Almeida, V.S. Braga, W.F. Pacheco, R.J. Cassella, Fluorescence determination of azithromycin in pharmaceutical formulations by using the synchronous scanning approach after its Acid derivatization, *J. Fluoresc.* 23 (1) (2013) 31–39.
- [68] P.Y. Khashaba, Spectrofluorimetric analysis of certain macrolide antibiotics in bulk and pharmaceutical formulations, *J. Pharm. Biomed. Anal.* 27 (6) (2002) 923–932.
- [69] K.P. Hunfeld, P. Kraiczy, T. Wichelhaus, V. Schäfer, V. Brade, New colorimetric microdilution method for in vitro susceptibility testing of *Borrelia burgdorferi* against antimicrobial substances, *Eur. J. Clin. Microbiol. Infect. Dis.* 19 (1) (2000) 27–32.
- [70] D. Haleem, E. Shireen, M. Haleem, W. Kaye, U. Bailer, G. Frank, A. Wagner, S. Henry, Degradation studies of azithromycin and its spectrophotometric determination in pharmaceutical dosage forms, *Pak. J. Pharm. Sci.* 19 (2) (2006) 98–103.

- [71] A.P. Kumar, J.H. Park, Azithromycin as a new chiral selector in capillary electrophoresis, *J. Chromatogr. A* 4 (9) (2011) 1314–1317.
- [72] M.V. Lebedeva, G.A. Bulgakova, A.F. Prokhorova, E.N. Shapovalova, M.G. Chernobrovkin, O.A. Shpigun, Azithromycin for enantioseparation of tetrahydrozoline in NACE, *Chromatographia* 76 (7–8) (2013) 375–379.
- [73] L. Miguel, C. Barbas, LC determination of impurities in azithromycin tablets, *J. Pharm. Biomed. Anal.* 33 (2) (2003) 211–217.
- [74] P. Zubata, R. Ceresole, M.A. Rosasco, M.T. Pizzorno, A new HPLC method for azithromycin quantitation, *J. Pharm. Biomed. Anal.* 27 (5) (2002) 833–836.
- [75] N. Kovačić-Bošnjak, J. Marincel, N. Lopotar, G. Kobrehel, Reversed-phase HPLC analysis of the semisynthetic macrolide antibiotic azithromycin, *Chromatographia* 25 (11) (1988) 999–1003.
- [76] Z.Y. Yang, L. Wang, X. Tang, Determination of azithromycin by ion-pair HPLC with UV detection, *J. Pharm. Biomed. Anal.* 49 (3) (2009) 811–815.
- [77] S. Pan, X. Xiao, Y. Wang, Y. Yin, X. Zhu, Comparison of HPLC and microbiological methods in the detection of azithromycin in eye drops [J], *Northwest Pharm. J.* 3 (2011) 011.
- [78] P. Matzneller, S. Krasniqi, M. Kinzig, F. Sörgel, S. Hüttner, E. Lackner, M. Müller, M. Zeitlinger, Blood, tissue and intracellular concentrations of azithromycin during and after end of therapy, *Antimicrob. Agents Chemother.* 28 (2013) 28.
- [79] A.D.H. Moreno, M.F.C.D. Silva, H.R.N. Salgado, Stability study of azithromycin in ophthalmic preparations, *Braz. J. Pharm. Sci.* 45 (2009) 219–226.
- [80] L.L. Brunton, L.L. Brunton; associate editors, B.A. Chaber, B.C. Knollmann Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 12th ed., 2011.
- [81] S.S. Martindale, *The complete drug reference*, 36th ed., Pharmaceutical press, London, 2009.
- [82] L.L. Brunton, H.P. Rang, M.M. Dale, J.M. Ritter, R.J. Flower, Rang & Dale *Pharmacology*, 6th ed., Churchill Livingstone 2011, 2007.
- [83] B. Moshiree, R. McDonald, W. Hou, P.P. Toskes, Comparison of the effect of azithromycin versus erythromycin on antroduodenal pressure profiles of patients with chronic functional gastrointestinal pain and gastroparesis, *Dig. Dis. Sci.* 55 (3) (2010) 675–683.
- [84] P. Chini, P.P. Toskes, S. Waseem, W. Hou, R. McDonald, B. Moshiree, Effect of azithromycin on small bowel motility in patients with gastrointestinal dysmotility, *Scand. J. Gastroenterol.* 47 (4) (2012) 422–427.
- [85] *Wikipedia*. [cited 2013 25/5]; Available from: [Wikipedia: http://en.wikipedia.org/wiki/Azithromycin](http://en.wikipedia.org/wiki/Azithromycin).
- [86] D.L. Hahn, Treatment of *Chlamydia pneumoniae* infection in adult asthma: a before-after trial, *J. Fam. Pract.* 41 (4) (1995) 345–351.
- [87] H. Noedl, S. Krudsood, K. Chalermratana, U. Silachamroon, W. Leowattana, N. Tangpukdee, S. Looreesuan, R.S. Miller, M. Fukuda, K. Jongsakul, S. Sriwichai, J. Rowan, H. Bhattacharyya, C. Ohrt, C. Knirsch, Azithromycin combination therapy with artesunate or quinine for the treatment of uncomplicated *Plasmodium falciparum* malaria in adults: a randomized, phase 2 clinical trial in Thailand, *Clin. Infect. Dis.* 43 (10) (2006) 1264–1271.
- [88] E. Gomez, M. Sanchez-Nunez, J.E. Sanchez, C. Corte, S. Aguado, C. Portal, J. Baltar, J. Alvarez-Grande, Treatment of cyclosporin-induced gingival hyperplasia with azithromycin, *Nephrol Dial Transplant* 12 (12) (1997) 2694–2697.
- [89] K. Hurt, D. Bilton, Cystic fibrosis, *Medicine* 40 (5) (2012) 273–276.
- [90] Joint Formulary Committee, *British National Formulary*, 57th ed., British Medical Association and Royal Pharmaceutical Society of Great Britain, London, 2009.