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Review of HPLC Methods for Determination of Azithromycin in Different Samples

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Abstract

Azithromycin is a semi-synthetic macrolide antibiotic of the azalide groups. It inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit of the bacterial 70S ribosome. It inhibits peptidyl transferase activity and interferes with amino acid translocation during the process of translation. Its effect may be bacteriostatic or bactericidal depending on the organism and the drug concentration. Azithromycin is one of the famous and important antibiotics agents and the determination methods of azithromycin in this article were tabulated with lots of chemical and instrumental methods that used in different parameters. Different high performance chromatographic methods have been reviewed in this paper.

Keywords Azithromycin, HPLC

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List of abbreviations: FIA = Flow injection analysis, FTIR = Fourier transform infra-red, GC = Gas chromatography, HPLC = High performance liquid chromatography, NMR = Nuclear magnetic resonance, UV = Ultraviolet

Introduction

High performance liquid chromatography (HPLC) is a procedure that is used for separation a mixture into its fractions or components. It is a separation method and the separated components identified by using any analytical method such as ultraviolet (UV)-visible, Fourier transform infra-red (FTIR), mass spectroscopy, nuclear magnetic resonance (NMR) etc. For quantitative analysis, the measurement of the area under the curve or peak height in the chromatogram is done. These bands or peaks are formed due to the separation of the compounds using different lengths on the chromatographic columns in HPLC and gas chromatography (GC) and on paper or thin layers in paper chromatography. The principle of HPLC is include that the samples are injected to flow by a mobile liquid phase via the particles of stable stationary phase. The compounds are separated into individual components based on their affinity towards the two phases during their flowing [(1)].

There are two types of separation in HPLC; isocratic elution, in which composition ratio of the mobile phase is keep same through the analysis and gradient elution, in which composition is subjected to change during the separation of sample [(2,3)]. The instrumentation of HPLC consists of mobile reservoir phase, pump, column, detector and recorder as shown in figure (1) [(4-7)].

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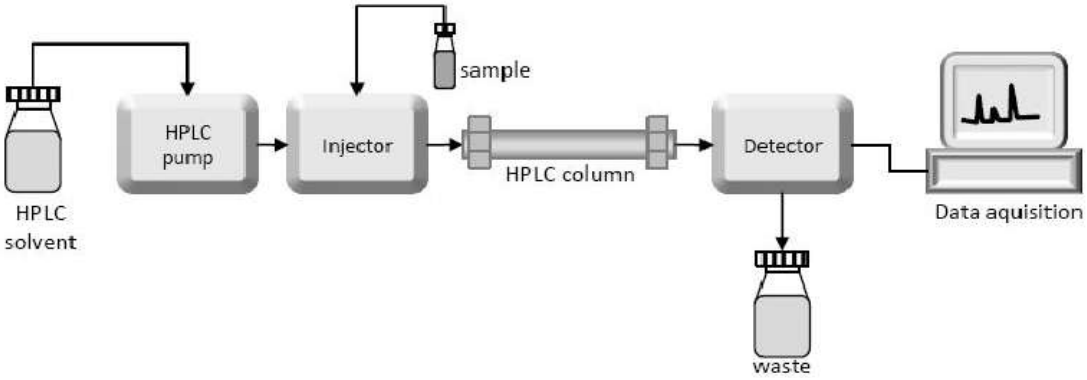

Figure 1. The instrumentation of HPLC [7].

The applications of HPLC are for qualitative and quantitative analysis, direct comparison method, calibration curve method, internal standard method, checking the purity of a compound, presence of impurities and determination of mixture of drugs [8,9].

Azithromycin is a 15-membered-ring macrolide that differs from erythromycin by the presence of a methyl-substituted nitrogen in the macrolide ring. Azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) as shown in figure (2) is derived structurally from erythromycin A by change the 9a carbonyl in the aglycone ring for a methyl-substituted nitrogen, and expansion of the ring to 15 members. This structural difference blocks the internal reaction to form the hemiketal, leaving acid hydrolysis of the ether bond to the neutral cladinose sugar as the main decomposition pathway. At 37°C and pH 2 with ionic strength = 0.02, azithromycin is hydrolyzed with 10% decay in 20.1 min, whereas the equivalent value for erythromycin is only 3.7 sec. The energy of activation for hydrolysis of the ether bond linking cladinose to azithromycin is about 25.3 kcal/mol; while, the internal dehydration reaction of erythromycin has an activation energy about 15.6 kcal/mol [10-15].

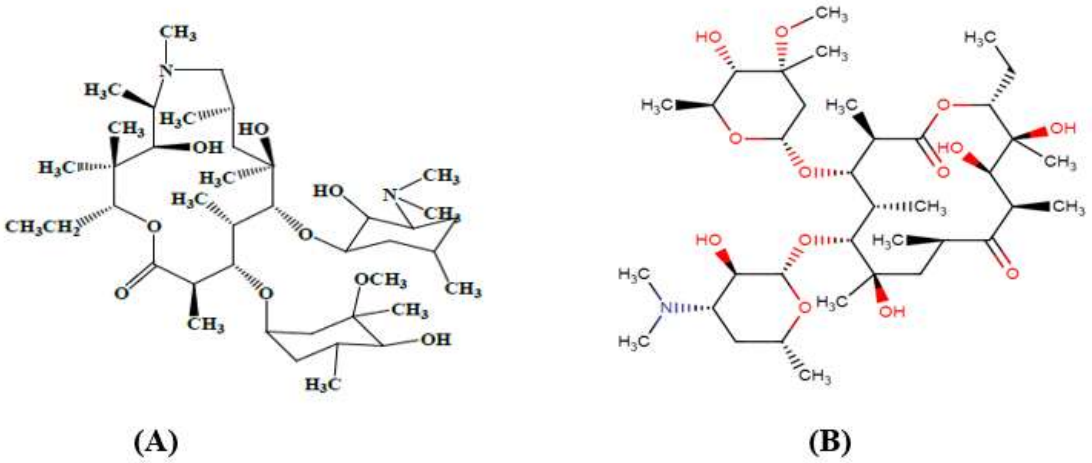

Figure 2. Chemical structure of Azithromycin(A) and Erythromycin(B) [12].

It is used against a variety of bacteria such as *Haemophilus influenzae*, *Streptococcus*, *pneumoniae*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *Mycobacterium avium* [16]. It has the ability to prevents these bacteria from growing by interfering proteins synthesis. According to differences in protein synthesis between bacteria and humans, these antibiotics do not interfere with production of proteins in humans [17]. It is a unique antibiotic that stays in the body for quite a while (has a longer half-life) allowing for once-a-day dosing and for shorter treatment courses for most infections [18]. Azithromycin is absorbed quickly after oral administration with a bioavailability about 36%. It has a various effect with food. It is also as a poorly water-soluble drug [19,20]. In this review article, we are tabulating the most recent HPLC methods that were used for determination of azithromycin in different formulations.

Methods and methodology of HPLC

The recent HPLC methods that were used for determination of azithromycin in pure, dosage forms and biological samples were based on efficiency on using the mobile phase and type of columns (stationary phase). These methods are summarized in table (1) including type of mobile phase, type of stationary phase, flow rate, retention time, linearity and detection limit [21].

Table 1. HPLC methods used for determination of Azithromycin

Mobile Phase	Stationary Phase	Flow rate	Retention Time	Linearity	Detection Limit	Ref.
Consist a mixture of 0.0335 M Phosphate Buffer (pH 7.5) and methanol in the proportion 20:80	C-8, 250 mm X 4.6 mm, 5 µm	1.2 ml/min	8.35 min	49.32-148.69 ppm	52.24 ppm	22
Consisting of Acetonitrile: Methanol: Phosphate buffer (40:40:20 v/v)	C18 (150×4.6 mm, 5 µm) column was used	1.0 ml/min	2.95 min	10-50 ppm	2.12 ppm	23
Methanol-phosphate buffer, pH 7.5 (80:20, v/v)	C18, 5 mm, 25 cm length, 4.6 mm	2.0 ml/min	--	0.3-2 mg/ml	0.0005 mg/ml	24
Ammonium acetate (0.05 M, pH 8.0) and acetonitrile (60:40, v/v)	250 x 4.6 mm, with 5 mm particle size and pore diameter 100 A, Boston pHlex ODS	0.8 ml/min	16 min	50.9-509.3 ppm	6.75 ppm	25
Buffer, acetonitrile and methanol (60:20:20) adjusted to pH 8.1 with phosphoric acid	C18 column	1.0 ml/min	5.23 min	--	--	26
Acetonitrile-0.1 M KH_2PO_4 (pH 6.5–0.1) M tetrabutyl ammonium hydroxide pH 6.5-water (25:15:1:59)	XTerra® (250 mm x 4.6 mm i.d., 5 µm particle size)	1.0 ml/min	8 min	50-150%	0.02%	27
It is consisting of acetonitrile, methanol, phosphate buffer, 0.05 M, pH 6.0 (20:20:60)	It is Eclipse XDB-CNTM 5 µm, 150 x 4.6 mm (Agilent Technologies, Palo Alto, CA) protected by a guard column Xterra RP18, 3.9x 20 mm	1.0 ml/min	16.6 min	10-400 ng/ml	--	28

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Consist of methanol, acetonitrile and phosphate buffer pH 8 (60:30:10)	It is C18 (250 mm × 4.6 mm i.d.)	1.0 ml/min	4.8 min	1-50 ppm	14.40 ng/ml	29
It is 35 mM ammonium acetate buffer (mobile phase-A) and acetonitrile and methanol in ratio of 90:10 (as mobile phase-B)	Luna C18 (3 µ, 2x150 mm) column	0.25 ml/min	7.8 min	0.5-50 ng/ml	--	30
Consist of methanol/buffer mobile phase at the ratio of 90:10	C18 column, 5 µm, 250 ×4.6 mm	1.5 ml/min	7.23 min	1-80 ppm	0.3 ppm	31
Contains Ammonium acetate solution (30 $mmolL^{-1}$, pH= 6.8) and acetonitrile (18:82, v/v)	It is Hypersil GOLD C-18 analytical column packed with deactivated silica (250 mm x 4.6 mm ID x 5 µm)	0.7 ml/min	7.95 min	5-200 ppm	0.476 ppm	32
Contains acetonitrile and phosphate buffer (pH 11 ± 0.05) of 60:40 (v/v)	It is Shodex ODP-50 column (250×4.6 mm i.d., 5 µm particles)	1.0 ml/min	7.34 min	--	--	33
Consisting of acetonitrile –2-methyl-2-propanol-hydrogenphosphate buffer, pH 6.5, with 1.5% triethylamine (33:7: up to 100, v/v/v)	End-capped ODB RP18 column	1.0 ml/min	12.83 min	0.25-15 ppm	0.37 ppm	34
it is acetonitrile –2-methyl-2-propanol–hydrogenphosphate buffer, pH 6.2, with 1.8% triethylamine (32:8: up to 100, v/v/v)	Using C18 ODB column (250×4.6 nm i.d.)	1.1 ml/min	12.35 min	0.004-4.8 mg/ml	0.02%	35
A mixture of Methanol and 0.0335M Phosphate Buffer (pH 7.5) (80:20 v/v)	It is ODS C18, 250 x 4.6 mm, 5 µm, L1 packing, column	1.2 ml/min	3.83 min	0.1-12 ppm	1.6 ppm	36
Ammonia buffer with pH = 6.7 (A) and acetonitrile (B)	Column Ascentis® with the length of 150 mm, inner diameter of 2.1 mm and particle size of 2.7 µm	0.6 ml/min	2.8 min	2.5-400 ng/ml	0.7 ng/ml	37

In general, several analytical techniques for the analysis of Azithromycin have been presented. However, further efforts to use widely modern chromatographic techniques HPLC coupled with tandem mass spectrometry for the quantitative analysis of Azithromycin. The main goals to be addressed in the future include improved selectivity, sensitivity, analytical simplicity, and efficiency of the HPLC method.

In conclusion, in this review article we conclude that Azithromycin can be determined in different samples using accurate analytical methods such as HPLC also these methods can be attached to different instruments such as flow injection analysis (FIA) and

spectrophotometry. We also found from the above summarized methods in table (1) that each method has it is advantaged like type of sample, cost of mobile and stationary phases beside the accurate of the obtained results. All the researchers can used any of these methods to qualitative and quantitative of azithromycin in it is sample.

References

1. Sabir AM, Molony M, Parminder SB. HPLC method development and validation: A review. Int Res J Pharm. 2013; 4(4): 39-46.
2. Bhardwaj SK, Dwivedi K, Agarwal DD. A review: HPLC method development and validation. Int J Analyt Bioanalyt Chem. 2015; 5(4): 76-81.
3. Vidushi Y, Meenakshi B. A review on HPLC method development and validation. Res J Life Sci Bioinform Pharmaceut Chem Sci. 2017; 2(6): 166-78. doi: 10.26479/2017.0206.12.
4. Sowjanya P, Subashini D, Rakesh S. A review on method development and validation by HPLC. Res Rev Pharm Pharmaceut Sci, 2015; 4(1): 42-50.
5. Hema, Reddy GS. A review on new analytical method development and validation by RP-HPLC, Int Res J Pharmaceut Biosci. 2017; 4(3): 41-50.
6. Xiao Y, Liu S, Gao Y, et al. Determination of antibiotic residues in aquaculture products by liquid chromatography tandem mass spectrometry: Recent trends and developments from 2010 to 2020. Separations. 2022; 9(2): 35. doi: https://doi.org/10.3390/separations9020035.
7. Rode DM, Rao NN. A review on development and validation of stability indication HPLC methods for analysis of acidic drugs. Int J Curr Pharmaceut Res. 2019; 11(4): 22-33. doi: https://doi.org/10.22159/ijcpr.2019v11i4.34939
8. Bhagat R, Saudagar.RB. A Review on analytical method development and validation. J Drug Delivery Therap. 2019; 9(3-s): 1064-7.
9. Kumar V, Bharadwaj R, Gupta G, et al. An overview on HPLC method development, optimization and validation process for drug analysis. Pharmaceut Chem J. 2015; 2(2):30-40.
10. McCarty JM. Azithromycin (Zithromax(R)). Infect Dis Obstet Gynecol. 1996; 4: 215-20.
11. McMullan BJ, Mostaghim M. Prescribing azithromycin. Aust Prescr. 2015; 38(3): 87-9. doi: 10.18773/austprescr.2015.030.
12. Lode H, Borner K, Koeppe P, et al. Azithromycin--review of key chemical, pharmacokinetic and microbiological features. J Antimicrob Chemother. 1996; 37 Suppl C:1-8. doi: 10.1093/jac/37.suppl_c.1.
13. Jelić D, Antolović R. From erythromycin to azithromycin and new potential ribosome-binding antimicrobials. Antibiotics (Basel). 2016; 5(3): 29. doi: 10.3390/antibiotics5030029.
14. Arayne SM, Sultana N, Shamim S, et al. Synthesis characterization and antimicrobial activities of azithromycin metal complexes. Mod Chem App. 2014; 2(3): 1-5. doi: 10.4172/2329-6798.1000133.
15. Whitman MS, Tunkel AR. Azithromycin and clarithromycin: overview and comparison with erythromycin. Infect Control Hosp Epidemiol. 1992; 13(6): 357-68. doi: 10.1086/646545.
16. Khan FA, Zahoor M, Islam NUI, et al. Synthesis of cefixime and azithromycin nanoparticles: an attempt to enhance their antimicrobial activity and dissolution rate. J Nanomat, 2016; 2016: 1-9.
17. Palanidamy P, Gowdhaman K, Jaykar B, et al. Formulation and evaluation of film coated tablets of azithromycin Usp. Int J Med Pharm. 2013; 1(1): 59-71.
18. Mohanty S, Mohapatra R, Patra S, et al. Formulation and in-vitro evaluation of azithromycin mouth dissolving tablets using super disintegrants. Res J Pharmaceut Biol Chem Sci. 2013; 4(3): 452-61.
19. Pouretedal HR. Preparation and characterization of azithromycin nanodrug using solvent/antisolvent method. Int Nano Lett. 2014; 4(1): 103-11.
20. Fiese EF, Steffen SH. Comparison of the acid stability of azithromycin and erythromycin A. J Antimicrob Chemother. 1990; 25 Suppl A: 39-47. doi: 10.1093/jac/25.suppl_a.39.
21. Waghule SN, Jain NP, Patani CJ, et al. Method development and validation of HPLC method for determination of azithromycin. Der Pharma Chemica, 2013; 5(4): 166-72.
22. Hinge MA, Bhavsar MM, Singh RD, et al. Spectrophotometric and high performance liquid chromatographic determination of cefpodoxime proxetil and azithromycin dihydrate in pharmaceutical formulation. Pharm Methods. 2016; 7(1): 8-16. doi: 10.5530/phm.2016.7.2.
23. Al-Rimawi F, Kharoaf M. Analysis of azithromycin and its related compounds by RP-HPLC with UV detection. J Chromatogr Sci. 2010; 48(2): 86-90. doi: 10.1093/chromsci/48.2.86.
24. Zeng A, Liu X, Zhang S, et al. Determination of azithromycin in raw materials and pharmaceutical formulations by HPLC coupled with an evaporative light scattering detector. Asian J Pharmaceut Sci. 2014; 9(2): 107-16. doi: https://doi.org/10.1016/j.ajps.2013.12.007.
25. Zubata P, Ceresole R, Rosasco MA, et al. A new HPLC method for azithromycin quantitation. J Pharm Biomed Anal. 2002; 27(5): 833-6. doi: 10.1016/s0731-7085(01)00554-4.
26. Okaru AO, Abuga KO, Kamau FN, et al. A Robust liquid chromatographic method for confirmation of drug stability of azithromycin in bulk samples, tablets and suspensions. Pharmaceutics. 2017; 9(1): 11. doi: 10.3390/pharmaceutics9010011.
27. Supattanapong S, Konsil J. Solid phase extraction and high performance liquid chromatography for the determination of azithromycin in human plasma.

Southeast Asian J Trop Med Public Health. 2008; 39(6): 978-87.

28. Singh AP, Chauhan I, Bhardwaj S, et al. HPLC method development and validation for Azithromycin in oral suspension. J App Pharmaceut Sci Res. 2019; 2(1): 7-12. doi: https://doi.org/10.31069/japsr.v2i1.2.

29. Ahmed MU, Islam MS, Sultana TA, et al. Quantitative determination of azithromycin in human plasma by liquid chromatography–mass spectrometry and its application in pharmackokinetic study, Dhaka Univ J Pharm Sci. 2012; 11(1): 55-63.

30. Ghari T, Kobarfard F, Mortazavi SA. Development of a simple RP-HPLC-UV method for determination of azithromycin in bulk and pharmaceutical dosage forms as an alternative to the USP method. Iran J Pharm Res. 2013; 12(Suppl): 57-63.

31. Abou Assi R, Darwis Y, Abdulbaqi IM, et al. Development and validation of a stability-indicating RP-HPLC method for the detection and quantification of azithromycin in bulk, and self-emulsifying drug delivery system (SEDDs) formulation. J App Pharmaceut Sci. 2017; 7 (09): 020-9.

32. Habibi B, Ghorbel-Abid I, Lahsini R, et al. Development and validation of a rapid HPLC method for multiresidue determination of erythromycin, clarithromycin, and azithromycin in aquaculture fish muscles. Acta Chromatographica. 2019; 31(2): 109-12. doi: https://doi.org/10.1556/1326.2017.00376.

33. Mahmoudi A, Boukhechem MS. Novel liquid chromatographic method for the simultaneous evaluation of erythromycin and azithromycin in human urine. J Mat Environ Sci. 2017; 8(6): 1953-9.

34. Mahmoudi A. LC determination and stability assessment of macrolide antibiotics azithromycin and spiramycin in bulk and tablet samples, Int Letters Natural Sci. 2015; 47: 1-10. doi: https://doi.org/10.18052/www.scipress.com/ILNS.47.1

35. Gajda A, Posyniak A, Błądek T. Analytical procedure for the determination of Tulathromycin in swine plasma. Bull Vet Inst Pulawy. 2013; 57: 191-5. doi: https://doi.org/10.2478/bvip-2013-0035T.

36. Bandapally C, Vamshi Y, Sasatry VG. Method development and validation of simultaneous estimation of azithromycin and dexamethasone in eye drops by RP-HPLC. Indo Am J Pharmaceut Res. 2018; 8(1): 1415-23.

37. Landová P, Vávrová M. A new method for macrolide antibiotics determination in wastewater from three different wastewater treatment plants. Acta Chimica Slovaca, 2017; 10(1): 47-53. doi: https://doi.org/10.1515/acs-2017-0008.

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