

Analytical Method Development and Validation for The Simultaneous Estimation of Azithromycin and Levofloxacin by RP-HPLC

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Abstract

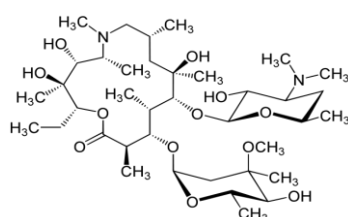
A new method was established for simultaneous estimation of Azithromycin and Levofloxacin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Azithromycin and Levofloxacin by using ZODIAC –SIL RP C18 column 4.6×100 mm 3.0µm, flow rate was 1.0 ml/min, mobile phase ratio was (75:25 v/v) acetonitrile:phosphate buffer (KH₂PO₄ and K₂HPO₄) pH 9 (pH was adjusted with orthophosphoric acid), detection wave length was 292 nm. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Azithromycin and Levofloxacin was found in concentration range of 6.25µg-37.5µg and 0.5µg-3.0µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.27 and 0.40, % RSD for intermediate precision was 0.27 and 0.94 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.17 and 6.60, and LOQ value was 0.032 and 0.1125 respectively.

Keywords

Azithromycin, Levofloxacin, RP-HPLC, Validation, Methanol.

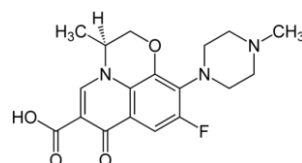
INTRODUCTION:

Azithromycin is an antibiotic medication used for the treatment of a number of bacterial infections. This includes middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections. Along with other medications, it may also be used for malaria.



Azithromycin

Levofloxacin, sold under the brand name Levaquin among others, is an antibiotic medication. It is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, H. pylori urinary tract infections, chronic prostatitis, and some types of gastroenteritis.



Levofloxacin

MATERIALS AND METHOD:

Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5μ) C-18 RP-column, Sonicator Analytical Technologies Limited-Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000⁺pH meter, Weighing machine

Chemicals

Azithromycin and Levofloxacin Potassium dihydrogen orthophosphate, Water and Methanol

for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Trial-6 (Optimized)

Chromatographic conditions

Column: Zodiac sil RP C18 4.6×100mm 3.0μm

Mobile phase ratio: ACN: pH 9buffer (75: 25 % v/v0

Detection wavelength: 292nm

Flow rate: 0.6ml/min

Injection volume: 20μl

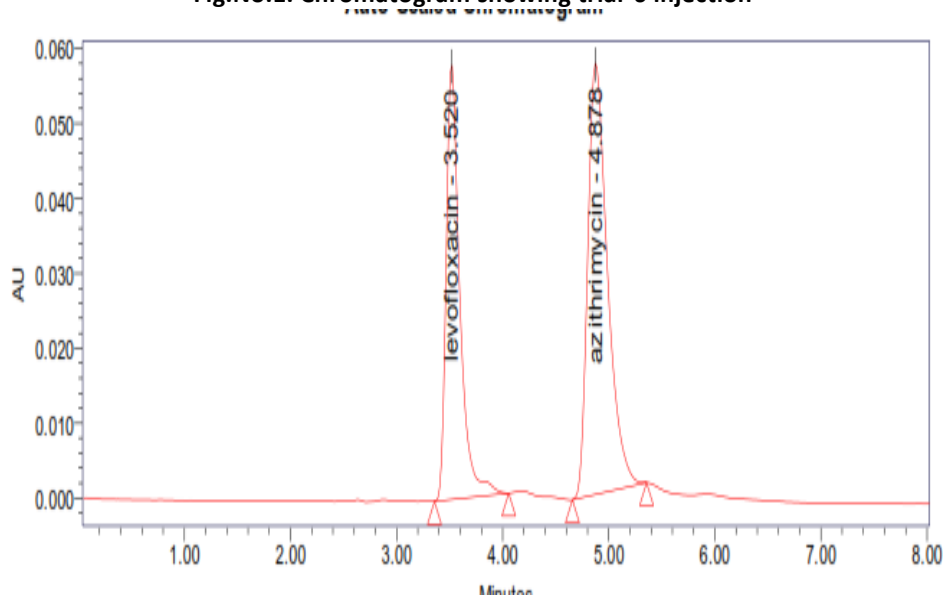
Column temperature: Ambient

Auto sampler temperature: Ambient

Run time: 4.5 min

Retention time: 3.520 and 4.878 mins.

Fig.No.1. Chromatogram showing trial-6 injection



S.No	Peak Name	R _t	Area	Height	USP plate count
1	Levofloxacin	3.520	10966728	1412054	3445
2	Azithromycin	4.878	1397231	177886	5441

Sample solution preparation

161.2 mg of Levofloxacin and Azithromycin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 1.5 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig. and results are tabulated in Table.

METHOD VALIDATION:

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

RESULTS AND DISCUSSION:

Linearity

Fig.2 Showing calibration graph for Levofloxacin

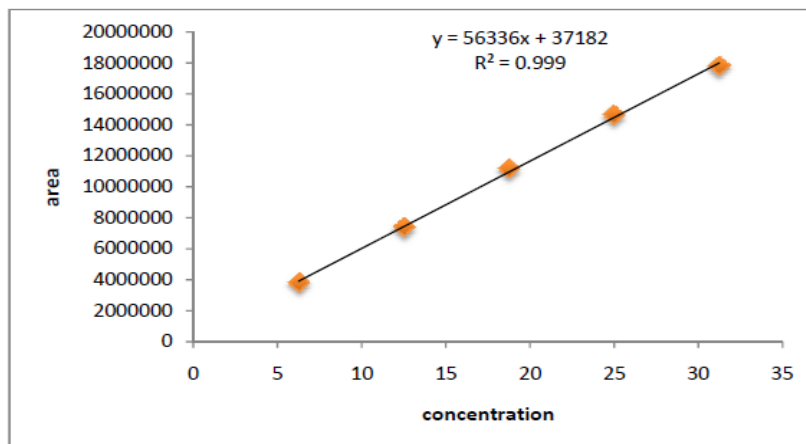
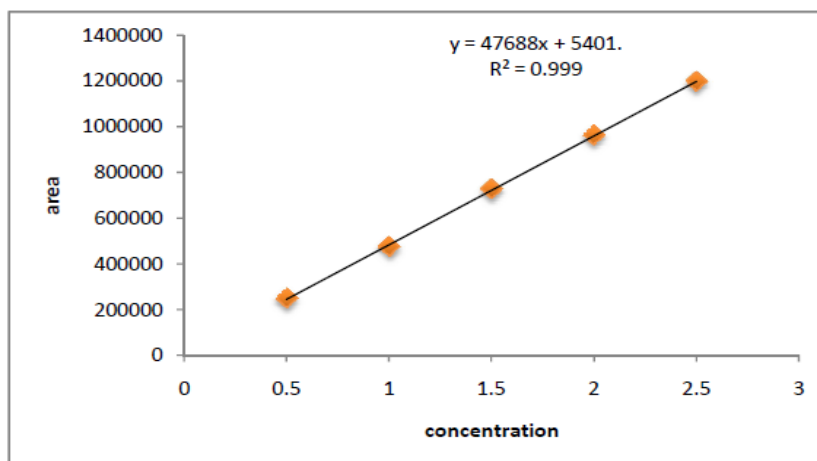


Fig.3 Showing calibration graph for Azithromycin



Accuracy

Table.No.1. Showing accuracy results for Levofloxacin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	7371253	5	4.9	99.91%	99.56%
100%	14634226.7	10	9.98	99.18%	
150%	2243270.7	15	14.89	99.60%	

Table.No.2. Showing accuracy results for Azithromycin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	5.0	4.9	99.53%	99.47%
100%	967998	10.0	9.59	99.38%	
150%	145437	15.0	14.85	99.52%	

Precision

Table.3 Showing % RSD results for Levofloxacin

	Peak Name	RT	Area($\mu\text{V}\cdot\text{sec}$)	Height(μV)	USP Plate Count	USP Tailing
1	Levofloxacin	3.517	520512	55970	3743.5	1.5
2	Levofloxacin	3.514	521717	56909	3777.3	1.5
3	Levofloxacin	3.513	521846	57014	3776.0	1.5
4	Levofloxacin	3.514	522710	56972	3822.2	1.5
5	Levofloxacin	3.514	528234	56798	3777.8	1.5
Mean			522013.9			
Std.Dev			1057.0			
%RSD			0.2			

Table .4 Showing %RSD results for Azithromycin

	Peak Name	RT	Area($\mu\text{V}\cdot\text{sec}$)	Height(μV)	USP Plate Count	USP Tailing
1	Azithromycin	4.864	787229	56504	3081.3	1.6
2	Azithromycin	4.864	789690	56992	3144.1	1.6
3	Azithromycin	4.863	790187	56962	3118.1	1.6
4	Azithromycin	4.862	791763	56934	3147.3	1.6
5	Azithromycin	4.862	794118	57105	3101.8	1.6
Mean			790597.2		3118.5	1.6
Std.Dev			2553.9			
%RSD			0.3			

Intermediate precision/Ruggedness

	Peak Name	RT	Area($\mu\text{V}\cdot\text{sec}$)	Height(μV)	USP Plate Count	USP Tailing
1	Levofloxacin	4.863	790742	56569	3075.9	1.6
2	Levofloxacin	4.860	794791	56512	3043.2	1.7
3	Levofloxacin	4.862	796445	56415	3049.7	1.6
Mean			793992.9			
Std.Dev			2934.1			
%RSD			0.4			

	Peak Name	RT	Area($\mu\text{V}\cdot\text{sec}$)	Height(μV)	USP Plate Count	USP Tailing
1	Azithromycin	3.513	521817	56358	3075.9	1.6
2	Azithromycin	3.515	522684	56384	3043.2	1.7
3	Azithromycin	3.516	522921	56456	3049.7	1.6
Mean			522473.9			
Std.Dev			581.3			
%RSD			0.1			

Detection limit

Table.5 Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(μg)
Levofloxacin	371827.90	563365963	2.17
Azithromycin	5401.60	479884400	0.0372

Quantitation limit

Table.6 Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(μg)
Levofloxacin	371827.90	563365963	6.60
Azithromycin	5401.60	479884400	0.112

Robustness

Table.7 Showing system suitability results for Levofloxacin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	3696	1.8
2	1.0	3646	1.4
3	1.2	3657	1.8

Table.No.8. Showing system suitability results for Azithromycin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.4	3108	1.8
2	0.5	3348	1.4
3	0.6	3057	1.9

Table.9 Showing system suitability results for Levofloxacin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3706	1.75
2	*Actual	3646	1.4
3	5 % more	3627	1.8

Table.No.10. Showing system suitability results for Azithromycin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3309	1.86
2	*Actual	3348	1.4
3	5 % more	3220	1.9

SUMMARY AND CONCLUSION:

This method is simple, specific, and easy to perform and requires short time to perform to analyse the samples. The developed method was validated in terms of Linearity, precision, robustness, LOD, LOQ and accuracy.

BIBLIOGRAPHY:

1. Shaikh Ka, Patil Sd et al. Simultaneous determination of azithromycin hydrochloride and chlorthalidone in pharmaceutical preparations by spectrophotometric, RP-HPLC, and HPTLC methods' JAOAC Int, 93(3), (2010), PP 904-10.
2. Surya Prakash Gupta., Development and validation of spectrophotometric, HPTLC and HPLC methods for the determination of Imipramine and Chlorthalidone in pharmaceutical dosage forms. Der Pharmacia Sinica, 3 (2), (2012), PP 185-192.
3. Fraga, J. Abizanda, A. Moreno, F. Leon, J. Simultaneous determination of imipramine and amitriptyline by derivative spectrophotometry. Journal of Pharmaceutical and Biomedical Analysis, Volume 9, issue 2, (1991), PP 109-115.
4. N.Karnakar,et al. Analytical Method Development and validation for the Simultaneous Estimation of Ephedrine and Theophylline by RP-HPLC Method. A.J.Med.pharm, sci.,2021,9(1):23-26.
5. Paduri Amani, Narendar Malothu, Karnakar N, Ramya sri S, RP-HPLC Method for Estimation of Zanamivir in API and Pharmaceutical Formulation. (2022) Int. J. Life Sci. Pharma Res.12(1): P20-27.
6. Prajapati, rajesh r parmar, dushyant a shah, "Development and validation of uv spectrophotometric area under curve for simultaneous estimation of diazepam and imipramine hydrochloride in pharmaceutical dosage form inventi rapid: pharm analysis and quality assurance, vol. 2013, article id- " inventi:ppaqa/1046/13 " , (2013).
7. Nuthalapati mamatha1*, B. Thangabalan1, S. Manohar Babu1Nuthalapati Mamatha. et al. / International Journal of Research in Pharmaceutical and Nano Sciences. 3(3), 2014, 200 – 214
8. M. Senthil Raja1*, Shan. S.Ha ., P. Perumala . rp-hplc method development and validation for the simultaneous estimation of azithromycin International Journal of Pharm Tech Research CODEN (USA): IJPRIF ISSN:0974-4304 Vol.2, No.1, pp 36-39, Jan-Mar 2010