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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AZITHROMYCIN AND LEVOFLOXACIN IN COMBINED TABLET DOSAGE FORM

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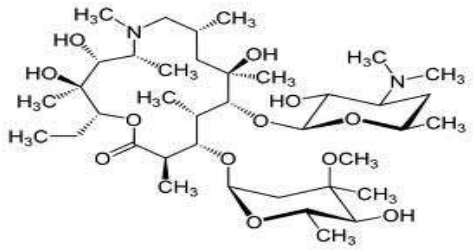
Abstract: An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Azithromycin and Levofloxacin in combined-dosage form. A Waters Symmetry Shielde Rp18, (250*4.6*5µ) column with mobile phase containing water pH 9.2 adjusted with di- Potassium hydrogen Phosphate: Methanol in the ratio of (60: 40, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 285 nm. The retention times of Azithromycin and Levofloxacin were 5.001min and 3.232min, respectively. The correlation co-efficient for Azithromycin and Levofloxacin was found to be 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Azithromycin and Levofloxacin in formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Azithromycin and Levofloxacin in combined dosage form.

Keywords: RP-HPLC, Azithromycin and Levofloxacin

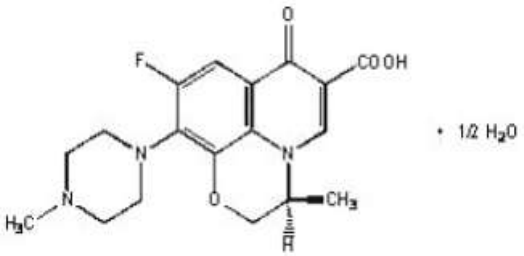
INTRODUCTION

Azithromycin is a macrolide anti biotic belonging to the azalide group. Chemically it is (2R,3S, 4R, 5R, 8R, 10R, 11R,12S,13S,14S)-11-((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2Hpyran-2-yloxy)-2-ethyl-3,4,10-trihydroxy-13-((2S,4R,5S)-5-hydroxy-4-methoxy-4hyltetrahydro-2H-Pyran-2-yloxy)-3,5,6,8,10,12,14-heptamethyl-1-oxa 6cyclopentade-can-5-one1, used asantibiotic and antibacterial.



Structure of Azithromycin

Levofloxacin hemihydrate is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe life-threatening bacterial infection or bacterial infection that have failed to respond to other antibiotic classes.IUPAC name is (S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylicacid.



Structure of Levofloxacin

MATERIAL AND METHODS

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software Waters Symmetry Shielde Rp18, (250*4.6*5µ).

Chemicals and Reagents Azithromycin and Levofloxacin was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. di- Potassium hydrogen Phosphate of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions: The mobile phase consisting of water (pH 9.2 adjusted with di- Potassium hydrogen Phosphate: Methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 285nm

and the run time was 6min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

Preparatio Of Standard Solution

Levofloxacin: Accurately weighed quantity, 500.0 mg of levofloxacin was transferred into 100ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15 min. make up the volume with mobile phase. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with mobile phase.

Azithromycin: Accurately weighed quantity, 500mg of Azithromycin was transferred into 100ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15mins make up the volume with mobile phase. Transferred above solution 5ml into 50ml volumetric flask and diluted to the mark with mobile phase.

Preparation Of Sample Solution: Accurately weighed 1370 mg of sampel. Transfer the sampel powder into 100ml of volumetric flask added 25ml of mobile phase and sonicated for 30mins and make up the volume with mobile phase and filtered through the 0.45µm filter paper Transfer above solution 5ml into 50 ml volumetric flask and make up the volume with mobile phase.

Method Validation

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc

Accuracy And Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table-3&4). The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Fig 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2).

Fig. 1: Standard chromatogram for Azithromycin and Levofloxacin

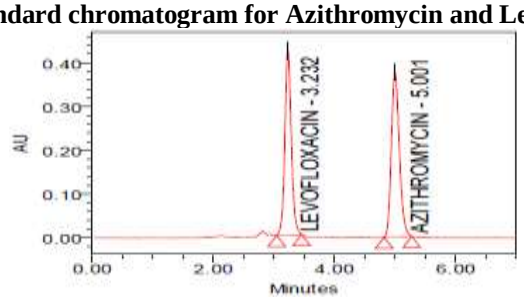


Fig. 2: Formulation chromatogram for Azithromycin and Levofloxacin

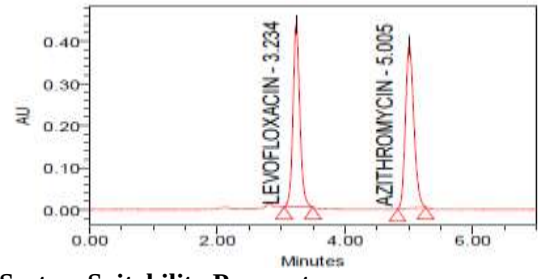


Table1: System Suitability Parameters

Parameters	Azithromycin	Levofloxacin
Correlation Coefficient	0.99	0.99
Regression Equation	y = 16616x	y = 19288x
LOD	6.1344	5.474
LOQ	20.4479	18.246
Theoretical plates	6790	4663
Tailing	1.193	1.088

Table 2 : Precision Studies

SNO	Sample Wt(mg)	Area(Levo)	Area(Azithr)	%Assya(lev)	%Assya(azi)
1	1370	3152466	3503557	99	100
2	1370	3151745	3505877	99	100
3	1370	3158954	3503969	99	100
4	1370	3157787	3508933	99	100
5	1370	3158663	3501948	99	100
6	1370	3155433	3501546	99	100

Fig. 3: Accuracy Chromatograms-50% of Azithromycin and Levofloxacin

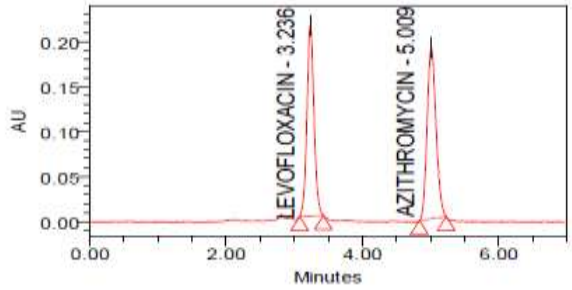


Fig. 4: Accuracy Chromatograms-100% of Azithromycin and Levofloxacin

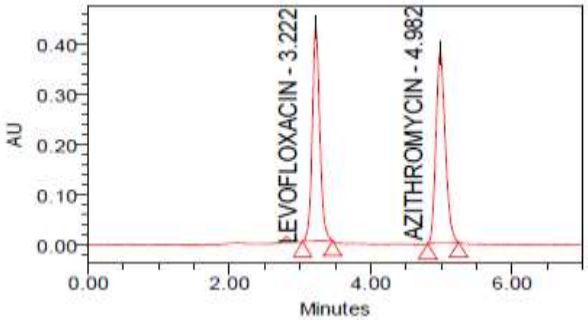


Fig. 5: Accuracy Chromatograms-150% of Azithromycin and Levofloxacin

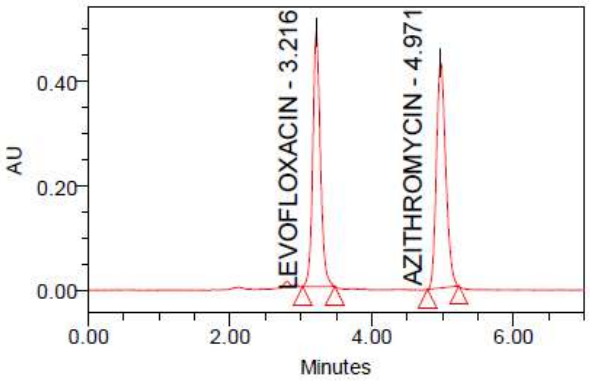


Table 3: Accuracy for Levofloxacin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	0.69	1579162	248.112	248.22	100	100
50%	0.69	1576213	248.112	247.75	100	
50%	0.69	1573695	248.112	247.36	100	
50%	0.69	1570450	248.112	246.85	99	
50%	0.69	1576050	248.112	247.73	100	
50%	0.69	1573005	248.112	247.25	100	
100%	1.37	3152275	496.224	495.48	100	100
100%	1.37	3151260	496.224	495.32	100	
100%	1.37	3157232	496.224	496.26	100	
150%	2.06	4726940	746.148	742.99	100	100
150%	2.06	4729848	746.148	743.45	100	
150%	2.06	4723140	746.148	742.39	99	
150%	2.06	4728536	746.148	743.24	100	
150%	2.06	4728895	746.148	743.30	100	
150%	2.06	4724036	746.148	742.53	100	

Table 4: Accuracy for Azithromycin

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	0.69	1751636	249.614	249.28	100	100
50%	0.69	1751270	249.614	249.23	100	
50%	0.69	1758366	249.614	250.24	100	
50%	0.69	1756333	249.614	249.95	100	
50%	0.69	1750913	249.614	249.18	100	
50%	0.69	1750708	249.614	249.15	100	
100%	1.37	3508264.00	499.229	499.27	100	100
100%	1.37	3509457.00	499.229	499.44	100	
100%	1.37	3506370.00	499.229	499.00	100	
150%	2.06	5253135	750.665	747.59	100	100
150%	2.06	5250130	750.665	747.16	100	
150%	2.06	5251467	750.665	747.35	100	
150%	2.06	5259637	750.665	748.51	100	
150%	2.06	5255756	750.665	747.96	100	
150%	2.06	5253042	750.665	747.57	100	

Linearity And Range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x (R^2=0.99) for Levofloxacin and y = 19288x (R^2=0.99) for Azithromycin The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Azithromycin and Levofloxacin shows in Fig 6 and the results for calibration curves are given in Fig 7&8.

Fig. 7: Linearity Curve for Levofloxacin

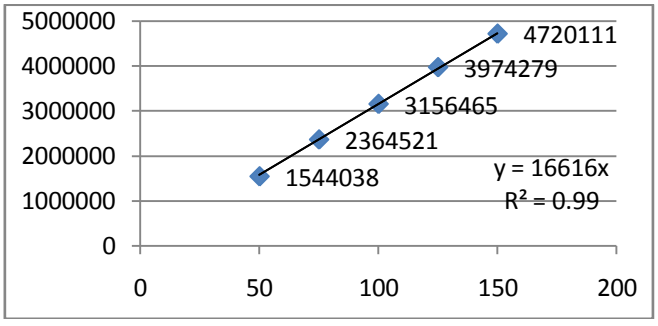


Fig. 8: Linearity Curve for Azithromycin

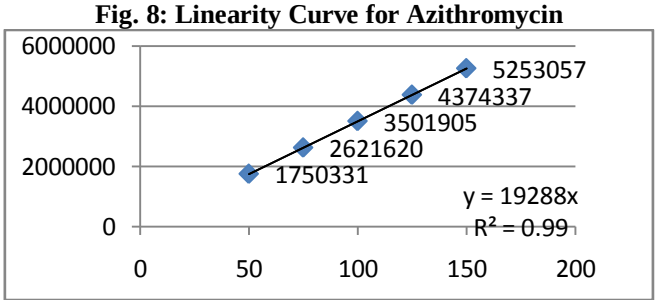
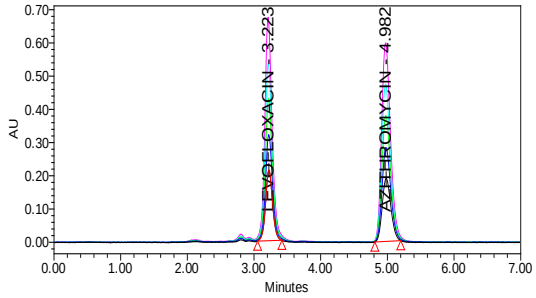
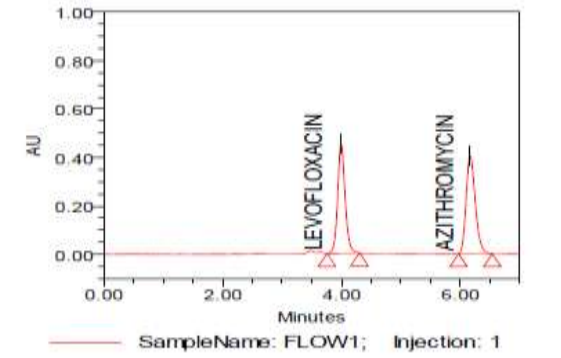


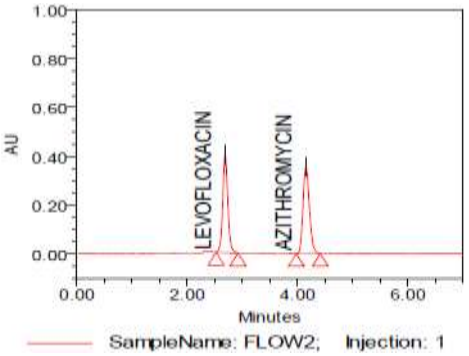
Fig. 9: Overlay chromatograms of Linearity for Azithromycin and Levofloxacin

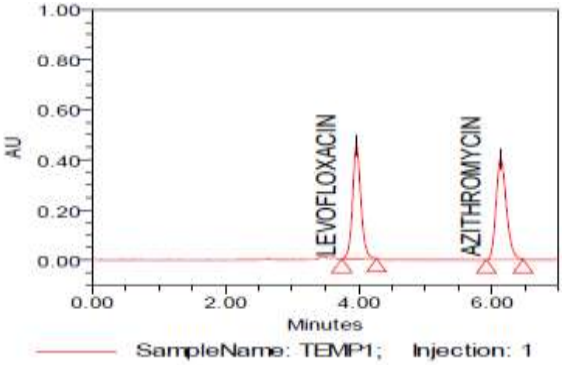


Robustness

Robustness of the method was determined by making slight changes in the chromatographi conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are robust (Table-5&6).







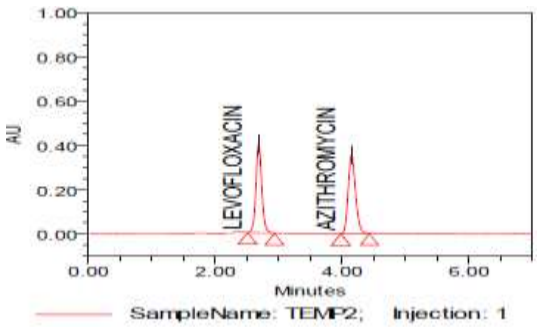


Table5:Robustness for Azithromycin

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT	S/N
1	TEMP-1	1	Azithromycin	6.130	4351835	1.210	7487	310.186
2	TEMP-2	1	Azithromycin	4.150	2874923	1.190	5954	241.709
3	FLOW-1	1	Azithromycin	6.165	4351433	1.243	7701	257.011
4	FLOW-2	1	Azithromycin	4.154	2883646	1.192	6211	186.216

Table6:Robustness for **Levofloxacin**

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT	S/N
1	TEMP-1	1	Levofloxacin	3.962	3923126	1.136	5106	347.299
2	TEMP-2	1	Levofloxacin	2.686	2592639	1.100	4348	273.369
3	FLOW-1	1	Levofloxacin	3.992	3941463	1.125	5203	287.179
4	FLOW-2	1	Levofloxacin	2.688	2582834	1.109	4342	208.905

LOD&LOQ: Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Azithromycin and Levofloxacin. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels.

(a) LOQ = 10 σ / S
(b) LOD = 3.3 σ / S
Where σ = residual standard deviation of response
S = slope of the calibration curve.

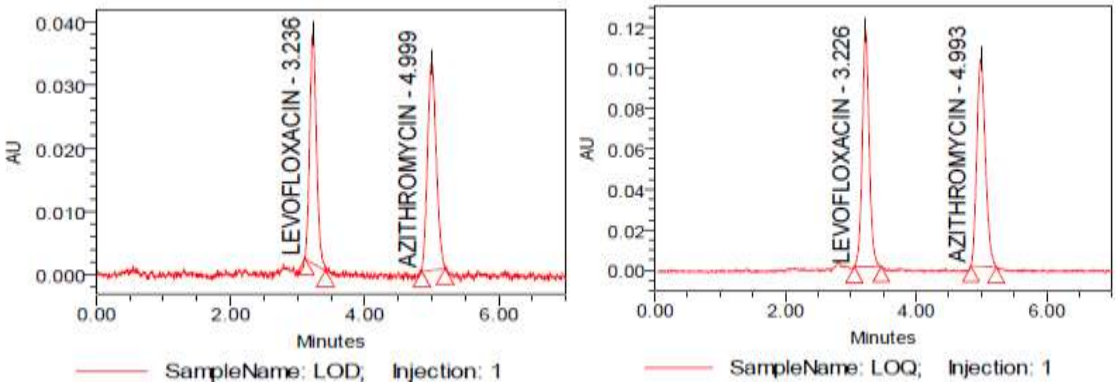


Table 7: LOD and LOQ For Azithromycin and Levofloxacin

	Sampel name	inj	Name	RT	Area
1	LOD	1	AZIT	4.999	299549
2	LOQ	1	AZIT	4.993	938141
1	LOD	1	LEVO	3.236	257745
2	LOQ	1	LEVO	3.226	863916

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Azithromycin and Levofloxacin standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Azithromycin and Levofloxacin studies. The method accuracy was evaluated by recovery studies. Azithromycin and Levofloxacin recovery was founded 100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given below fig: 7&8 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co-efficient.Linear correlation was found to be Y= 16616 for Levofloxacin and y = 19288 for Azithromycin Method robustness results were given by table 5&6, LOQ and LOD Rsults were given by table 7.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Azithromycin and Levofloxacin in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Azithromycin and Levofloxacin in pure and its pharmaceutical dosage forms.

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REFERENCES

1. USP 22/NF 17. General chapters<1649>, NF Monographs. Rockville MD. United states Pharmacopoeia convention. **2006**; 1710-12.
2. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization,IFPMA, Geneva, 2003 Indian Pharmacopoeia-2007. General chapters<p.No.78> Monographs. United states Pharmacopoeia convention. **2006**.
3. Monika Bakshi, Saranjit Singh., Development of validated stability indicating assay methods-critical review, *Journal of Pharmaceutical and Biomedical Analysis*, **2002**; 28(6); 1011-1040.
4. Reynolds, D.W., Facchine, K.L., Mullaney, J.F., Alsante, K.M., Hatajik, T.D., Motto, M.G., Available guidance and best practices for conducting forced degradation studies, *Pharm Tech*, **2002**; 48-56.
5. FDA Guidance for Industry, Analytical Procedures and Methods Validation (draft guidance), August **2000**.
6. T. Kumar, A. Chitra, V. Amrithraj, and N. Kumar, "New RP-HPLC method development and validation for estimation of levofloxacin in tablet dosage form," Journal of Global Trends in Pharmaceutical Sciences, **2011**; 2(3); 264–276,
7. K. Kothekar, J. Balasundaram, A. Khandhar, and R. Mishra, "Quantitative determination of levofloxacin and ambroxol hydrochloride in pharmaceutical dosage form by reversed-phase high performance liquid chromatography," *Eurasian Journal of Analytical Chemistry*, **2007**; 2.
8. T. Santhoshi, K. Kumar, V. Rao, and A. Ravipati, "Development and validation of RP-HPLC method for simultaneous estimation of levofloxacin and ornidazole in pharmaceutical dosage form," *Journal of Pharmacy Research*, **2011**; 4(11): 38-64.
9. N. S. Lakka and N. Goswami, "A novel isocratic RP-HPLC method development and validation for estimation of 5HMF in Levofloxacin Hemihydrate intravenous infusion," *International Journal of Research in Pharmaceutical Sciences*, **2011**; 2(1): 45–51.
10. U. Neckel, C. Joukhadar, M. Frossard, W. Jäger, M. Müller, and B. X. Mayer, "Simultaneous determination of levofloxacin and ciprofloxacin in microdialysates and plasma by high-performance liquid chromatography," *Analytica Chimica Acta*, **2002**; 463(2); 199–206,
11. J. Mehta, Y. Pancholi, V. Patel, N. Kshatri, and N. Vyas, "Development and validation of a sensitive stability indicating method for quantification of levofloxacin related substances and degradation products in pharmaceutical dosage form," *International Journal of PharmTech Research*, **2010**; 2(3): 1932–1942.
12. M. Green, a Practical Guide to Analyticalmethod validation; Analytical chemistry news and features, 1, **1996**; 309A.
13. Chen X, Bates, Morris KR. *Journal of Pharmaceutical biomedical analysis*, **2001**; 26-63.
14. Gombas A, Antal, Szabo, Marton. S. Eros.I, *International Journal of pharmaceuticalanalysis*, **2003**, 256: 25.
15. 15.Kamau F.N, Naugi. J.K, Roets. E. and Chepkwony, H.K. "Isocratic liquid chromatographic method for the analysis ofAzithromycin in Bulk sample". *Journal of Chromatographic science*. **2002**; 24(4): 529-533.
16. Biljana Nigovic and Branimir Simunic."Voltammetric assay of Azithromycin in pharmaceutical dosage forms". *Journal of pharm Biomed Anal*. **2003**; 27(6): 115-120.