INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

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

The aim of the present study is to develop a simple, accurate, precise, sensitive, less expensive and less time consuming method RP-HPLC for simultaneous determination of azithromycin and lovofloxacinin combined tablet dosage form. The method was validated for parameters like accuracy, linearity, precision, specificity, robustness and system suitability. The column efficiency has determined is not less than 3000 USP plate count and tailing factor is not more than 2.0. The %Relative standard deviation for the peak areas of the 6 replicate injections is not more than 2.0%. The % RSD of assay of 6 replicate injections was found to be within the limits. The recovery results indicating that the test method has an acceptable level of accuracy. The correlation coefficient met the acceptance criteria of NLT 0.999. The LOD and LOQ values from the study demonstrate that the method is sensitive. The system suitability parameters found to be within the limits for a change in temperature and flow rateand from the results it is concluded that the method is Robust.

Keywords: RP-HPLC, Azithromycin and Levofloxacin.

INTRODUCTION

Azithromycin is a macrolide anti biotic belonging to the azalide group and it is used as antibiotic and antibacterial. The IUPAC name of the drug azithromycin is (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-11-{[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-2-ethyl-3,4,10-trihydroxy-13-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,6, 8,10, 12,14-heptamethyl-1-oxa-6-aza cyclopenta decan-15-one (Fig.1).

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 has failed to respond to other antibiotic classes. IUPAC name is (S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl piperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylicacid. (Fig. 2).

Literature survey revealed few analytical techniques for the estimation of azithromycin[1,2] and levofloxacin[3-8] individually or in combination with other drugs but limited number of methods are available for the determination of these drugs in combination. So the aim of the present study is to develop a simple and specific RP-HPLC method for simultaneous determination of these two drugs in bulk and dosage form.

MATERIAL AND METHODS

Instrumentation

The separation was carried out on Waters HPLC system 2695 alliance with binary pump, Waters

2998 PDA detector, Waters Empower2 software Thermo hypersil BDS C18 (150mm×4.6mm×5µ).

CHEMICALS AND REAGENTS

Azithromycin and levofloxacin was a gift sample by LARA Laboratories Ltd., Hyderabad. Methanol of HPLC grade purchased from E. Merck (India) Ltd., Mumbai.

HPLC Conditions

The mobile phase consisting of orthophosphoric acid (pH 2.4±0.05): Methanol (HPLC grade) was filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 40:60v/v was pumped into the column at a flow rate of 0.8ml/min. The column temperature was maintained at 30°C. The detection was monitored at 269nm with a run time of 6 min. The volume of injection loop was 5µl prior to injection of the drug solution the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Preparation of Standard Solution

Accurately weighed and transferred100 mg of azithromycin and 100mg of levofloxacin Standards into a 100 mL clean dry volumetric flask, and added about 20 mL of solvent mixture sonicated to dissolve and the volume was made up to the mark. Then transfer 5mL above solution into 25 mL volumetric flask and dilute with solvent (200µg/ml).

Preparation of Sample Solution

Weighed and finely powdered 20 tablets. The tablet powder equivalent to 176.760 mg was transferred into 100 mL clean, dry, volumetric flask. Then 20 mL of solvent was added to dissolve and sonicated for 30 min with occasionally shaking. The volume was made up to the mark with solvent mixture. Then transferred 5.0 mL above solution into 25 mL volumetric flask and diluted with solvent mixture.

Method Validation

System Suitability Studies

For system suitability, six replicates of standard solutions of azithromycin and levofloxacin were injected and studied the suitability parameters like plate number (N), resolution (R), retention time (α), tailing factor and %RSD were studied with the help of standard chromatograms (Table1). 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. Twenty µl each of standard and sample solution was injected into optimized chromatographic conditions and no interference was observed with the analyte peaks so the method is specific (Fig. 3 and 4).

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-2 and 3). For precision the sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits and results are tabulated in Table No. 4.

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 (R2=0.99) for levofloxacin and y = 19288x (R2=0.99) for azithromycin. The results show that an excellent correlation exists between areas and concentration of drugs within the concentration range. The results for calibration curves are given in Fig. 5 and 6.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic 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 and 6).

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). The results obtained are presented in the table 7.

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

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters like retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1. So we can say that system is suitable for analysis. Method specificity was concluded by fig:2 and fig:3, 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 4 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 %RSD was very low so method is accurate shown in table 2 and 3. Linearity calibration curve was given below fig: 3 and 4 and plot the graph with 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 and 6, LOQ and LOD Results were given by table 7 and 8.

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 easilyand conveniently adopt for routine quality control analysisof azithromycin and levofloxacin in pure and its pharmaceutical dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Acme Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of azithromycin and levofloxacin for research. The authors are highly thankful to Principal and Management of Malla Reddy Institute of Pharmaceutical sciences for providing all the facilities to carry out the work.

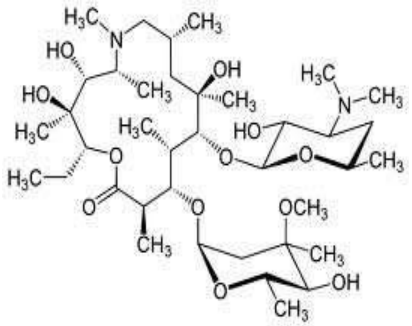

Fig. 1: Structure of Azithromycin

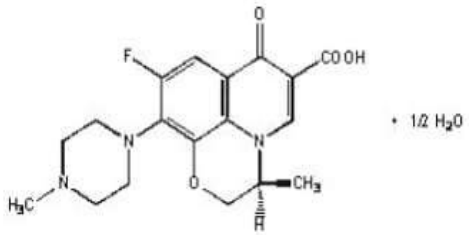

Fig. 2: Structure of Levofloxacin

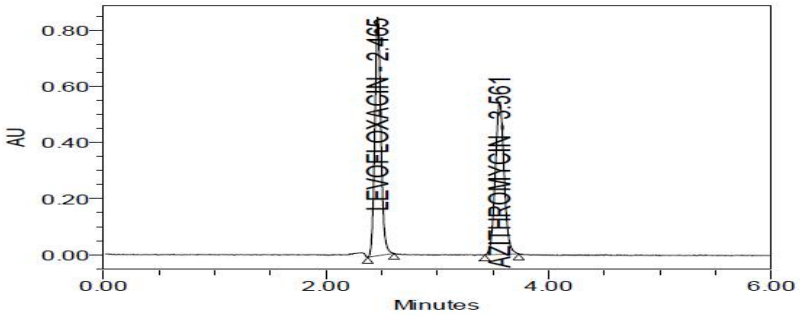

Fig. 3: Standard chromatogram for Azithromycin and Levofloxacin

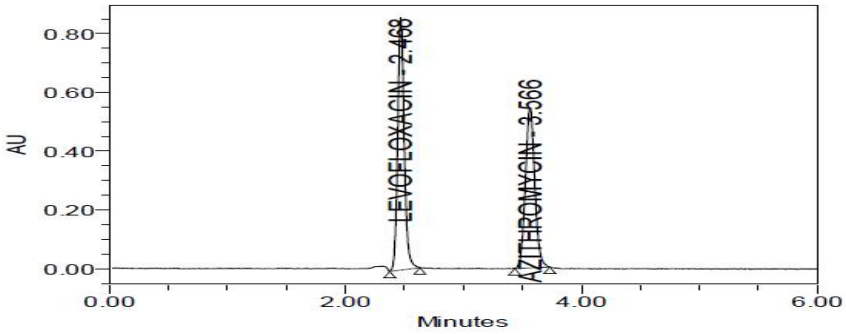

Fig. 4: Sample chromatogram for Azithromycin and Levofloxacin

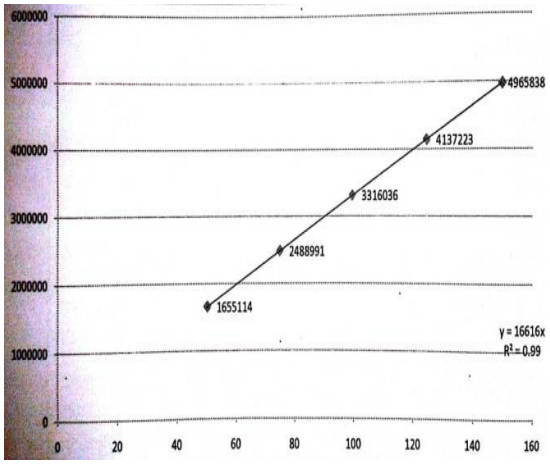

Fig. 5: Linearity Curve for Levofloxacin

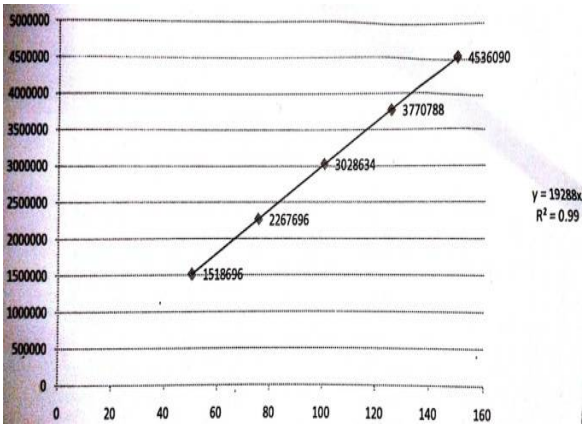

Fig. 6: Linearity Curve for Azithromycin

Table 1: System Suitability Parameters

Parameters	Levofloxacin	Azithromycin
Retention time (min)	2.465	3.561
Tailing	1.10	1.06
Resolution	.....	8.87
Theoretical plates	10105	9836
%RSD	0.4%	0.5%

Table 2: Accuracy for Levofloxacin

LEVOFLOXACIN						
Spiked Level	**Sample Weight (mg)**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	88.38	1652030	99.00	988.67	99.79	99.96
50%	88.38	1657674	99.00	992.05	100.20	
50%	88.38	1655411	99.00	990.69	100.02	
50%	88.38	1650955	99.00	988.03	99.75	
50%	88.38	1650096	99.00	987.51	99.69	
50%	88.38	1654105	99.00	989.91	99.84	
100%	176.76	3313545	198.00	1983.02	100.15	100.07
100%	176.76	3316652	198.00	1981.88	100.05	
100%	176.76	3319052	198.00	1981.31	100.03	
150%	265.14	4964741	297.00	2971.19	100.03	100.01
150%	265.14	4962880	297.00	2970.08	100.01	
150%	265.14	4966332	297.00	2972.14	100.06	
150%	265.14	4965866	297.00	2971.86	100.03	
150%	265.14	4963512	297.00	2970.46	100.01	
150%	265.14	4962139	297.00	2969.63	99.96	

Table 3: Accuracy for Azithromycin

AZITHROMYCIN						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	88.380	1519407	100.000	100.254	100.2	100.03
50%	88.380	1516453	100.000	100.059	100.02	
50%	88.380	1518356	100.000	100.184	100.1	
50%	88.380	1518668	100.000	100.205	100.2	
50%	88.380	1512021	100.000	99.766	99.76	
50%	88.380	1514881	100.000	99.955	99.95	
100%	176.760	3027419	200.000	199.755	99.85	100.12
100%	176.760	3027863	200.000	199.785	100.86	
100%	176.760	3021434	200.000	199.360	99.65	
150%	265.140	4536404	300.000	299.321	99.73	99.72
150%	265.140	4534724	300.000	299.210	99.71	
150%	265.140	4539500	300.000	299.525	99.83	
150%	265.140	4536102	300.000	299.301	99.72	
150%	265.140	4531223	300.000	298.979	99.63	
150%	265.140	4533283	300.000	299.115	99.70	

Table 4: Precision Studies

S.No	Sample Weight	Levofloxacin	Azithromycin	% Assay	% Assay
1	176.760	3318410	3028194	99.87	100.01
2	176.760	3317064	3025939	99.88	100.02
3	176.760	3319381	3020519	99.95	100.06
4	176.760	3312404	3025427	100.02	99.98
5	176.760	3313933	3020646	100.05	99.99
6	176.760	3318844	3026929	99.96	99.98
Average				99.95	100.0
SD				0.06	0.02
%RSD				0.09	0.11

Table 5: Robustness for Levofloxacin

Peak Name: LEVOFLOXACIN

	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	TEMP1	LEVOFLOXACIN	2.466	3132526	1.14	10092
2	TEMP2	LEVOFLOXACIN	2.462	3200980	1.17	10350
3	FLOW1	LEVOFLOXACIN	3.279	4109676	1.10	10142
4	FLOW2	LEVOFLOXACIN	1.976	2541455	1.15	9238

Table 6: Robustness for Azithromycin

Peak Name: AZITHROMYCIN

	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count	USP Resolution
1	TEMP1	AZITHROMYCIN	3.561	2903872	1.08	9621	8.85
2	TEMP2	AZITHROMYCIN	3.557	2929022	1.12	9710	8.89
	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count	USP Resolution
3	FLOW1	AZITHROMYCIN	4.741	3842432	1.06	10483	8.86
4	FLOW2	AZITHROMYCIN	2.870	2350551	1.09	8796	8.52

Table 7: LOD and LOQ for Azithromycin and Levofloxacin

Peak Name: LEVOFLOXACIN

	SampleName	Peak Name	RT	Area
1	LOD	LEVOFLOXACIN	2.468	724557
2	LOQ	LEVOFLOXACIN	2.468	1706753

Peak Name: AZITHROMYCIN

	SampleName	Peak Name	RT	Area
1	LOD	AZITHROMYCIN	3.570	666185
2	LOQ	AZITHROMYCIN	3.566	1578224

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