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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-tablet dosage form. A water Symmetry ACE_5Rp18, (150*4.6*5µ) column with mobile phase containing 0.1% ortho phosphoric acid: Methanol in the ratio of (60:40v/v) was used. The flow rate was 1.0mL/min, column temperature was 30°C and effluents were monitored at 280nm. The retention times of Azithromycin and Levofloxacin were 3.623 min and 2.401min, respectively. The correlation co-efficient for Azithromycin and Levofloxacin were 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.

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INTRODUCTION

Azithromycin (Figure No.1) is a widely used macrolide anti biotic and belongs to azalide group. Its chemical formula is (2R,3S, 4R, 5R, 8R, 10R,11R,12S,13S,14S)-11-((2S, 3R, 4S, 6R)-4 (di methyl amino) -3- hydroxy-6-methyltetrahydro-2Hpyran-2-yloxy)-2-ethyl-3,4,10-trihydroxy-13((2S, 4R,5S)-5-hydroxy- 4- methoxy- 4 hyltetrahydro-2H Pyran -2-yloxy)-3, 5, 6, 8, 10, 12, 14 - heptamethyl-1-oxa6cyclopentade-can-5-one[1].

Levofloxacin hemihydrate, a synthetic chemotherapeutic antibiotic, belongs to

fluoroquinolone drug class and is used to treat a lot of life-threatening bacterial infections or infections that have developed ressistance to other antibiotic classes. The IUPAC name of levofloxacin hemihydrate 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-carboxylic acid.

MATERIALS AND METHODS

Instrumentation

The separation was carried out on HPLC system. It was done with Waters 2695 alliance with binary HPLC pump, Waters2998 PDA detector, Waters Empower2 software and Waters Symmetry ACE_5Rp8, (150*4.6*5µ).

Chemicals and Reagents

Levofloxacin and azithromycin, Methanol of HPLC grade, Orthophorsphoric acid of AR grade.

HPLC Conditions

Methanol (HPLC grade) and mobile phase consisting of 0.1% ortho phosphoric acid were filtered through 0.45µ membrane filter prior to use. Before pumping from the solvent reservoir they were degassed. In the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was maintained at 30°C. The detection was monitored at 280nm and the runtime was 6min. Volume of injection loop was 10µl. prior to injection of the drug solution; the column was equilibrated for about 15min. with the mobile phase flowing through the system (Figure No.3 and 4).

Preparation of Standard Solution

Levofloxacin

500.0 mg of accurately weighed levofloxacin was transferred into 100ml volumetric flask and added 30ml of mobile phase and sonicated for 15 min. The volume was made up with mobile phase. Transferred 5ml of above solution into 25 ml volumetric flask and diluted to the mark with mobile phase.

Azithromycin

500mg of accurately weighed Azithromycin was transferred into100ml volumetric flask and added 30ml of mobile phase and sonicated for 15mins. The volume was made up with mobile phase. Transferred 5ml of above solution into 25ml volumetric flask and diluted to the mark with mobile phase.

Specificity

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

Accuracy and Precision

The methods accuracy and precision was determined by recovery experiments the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained. The recovery studies were conducted six times for precision.

Preparation of Sample Solution

The sample was accurately weighed. It was transferred to a 100ml volumetric flask and added 25ml of mobile phase and then sonicated for 30mins. It was made up to the volume with mobile phase and filtered through the 0.45µm filter paper. 5ml of the above solution was transferred into 25ml volumetric flask and made up to the volume with mobile phase.

Method Validation

System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table No.1). The suitability of the system for the analysis of this drug combination was demonstrated by the values obtained. The system suitability parameters may fall within ±3% standard deviation range during routine performance of the method. Standard drugs added recoveries were found to be accurate (Table No.3 and 4). Inter-day and intra-day variation method was used to demonstrate precision of the studies done. Six repeated injections in intraday studies of standard and sample solutions revealed the response factor of drug peaks and percentage RSD. 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. Chromatograms of three different levels were shown in Figure No.5, 6 and 7. The data obtained proved the developed RP-HPLC method to be precise (Table No.2).

Linearity and Range

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=43363x (R^2=0.99) for levofoloxicin and y=55207x (R^2=0.99) for Azithromycin. The results show an excellent correlation existed between areas and concentration of drugs within the concentration range as indicated above. The overlay chromatograms of Linearity for Azithromycin and Levofloxacin were shown in Figure No.8 and results for calibration curves are given in Figure No.8 and 9.

Robustness

This methods robustness was determined by making a little change in the chromatographic conditions. No marked changes were observed in the chromatograms, demonstrating that the RPHPLC method developed was robust (Table No.5 and 6).

LOD and LOQ

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

(a) LOQ=10σ /S

(b) LOD=3.3σ / S

Where

σ =residual standard deviation of response

S=slope of the calibration curve.

RESULTS AND DISCUSSION

Table No.1 gives system suitability results and uniformity was shown in suitability parameters like retention time, resolution, tailing plate count. The % RSD was less than1 by which we can say that system is suitable for analysis method. Figure No.1 and Figure No.2 concluded systems specificity. They were figures of Azithromycin and Levofloxacin standard chromatograms and other one is formulation. Placebo was not observed and excipients peaks interference with standard and analytic peaks of it proves method is selective. Table No.2 result shows 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 .thus the method is accurate, shown in Table No.3 and 4. Linearity calibration curve was given in Figure No.8 and 9 and 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=43363 for Levofloxacin and y=55207 for Azithromycin method robustness results were given by Table No.5 and 6, LOQ and LOD Results were given by Table No.7 and Figure No.10.

Table No.1: System Suitability Parameters

S.No	Parameters	Azithromycin	Levofloxacin
1	Correlation Coefficient	0.99	0.99
2	Regression Equation	Y=55207x	Y=43363x
3	LOD	3.16	3.04
4	LOQ	10.44	9.37
5	Theoretical plates	6590	3473
6	Tailing	0.85	1.20

Table No.2: Precision Studies

S.No	Sample Weight	Sample Area-1	Sample Area-2	% Assay	% Assay
1	1387.10	2394390	5511941	99	100
2	1387.10	2397335	5506733	99	100
3	1387.10	2390818	5512638	99	100
4	1387.10	2399934	5549693	99	100
5	1387.10	2398086	5538321	99	100
6	1387.10	2393871	5515591	99	100
Average Assay	---	---	---	99	100

Table No.3: Accuracy for Levofloxacin

S.No	Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	Mean
1	50%	693.55	1193969	495.00	494.77	100	98
2	50%	693.55	1179927	495.00	488.95	99	
3	50%	693.55	1142653	495.00	473.51	96	
4	50%	693.55	1179927	495.00	484.87	99	
5	50%	693.55	1142653	495.00	479.42	97	
6	50%	693.55	1193969	495.00	494.88	96	
7	100%	1387.10	2399683	990.00	994.41	100	100
8	100%	1387.10	2393869	990.00	992.00	100	
9	100%	1387.10	2394705	990.00	992.35	100	
10	150%	2080.69	3539047	1485.00	1466.55	99	100
11	150%	2080.69	3597893	1485.00	1490.94	100	
12	150%	2080.69	3546409	1485.00	1469.60	99	
13	150%	2080.69	3597893	1485.00	1470.69	100	
14	150%	2080.69	3538041	1485.00	1484.50	99	
15	150%	2080.69	3586489	1485.00	1487.49	100	

Table No.4: Accuracy for Azithromycin

S.No	Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	Mean
1	50%	693.55	2782839	500.000	502.96	101	100
2	50%	693.55	2748603	500.000	496.78	99	
3	50%	693.55	2728800	500.000	493.20	99	
4	50%	693.55	2733480	500.000	502.87	100	
5	50%	693.55	2804408	500.000	489.42	99	
6	50%	693.55	2718450	500.000	494.88	99	
7	100%	1387.10	5511418	1000.00	996.12	100	100
8	100%	1387.10	5514453	1000.00	996.67	100	
9	100%	1387.10	5516090	1000.00	996.97	100	
10	150%	2080.69	8242819	1500.00	1489.79	99	99
11	150%	2080.69	8225743	1500.00	1486.70	99	
12	150%	2080.69	8214188	1500.00	1484.62	99	
13	150%	2080.69	8274080	1500.029	1491.69	99	
14	150%	2080.69	8247892	1500.00	1494.50	99	
15	150%	2080.69	8254790	1500.029	1497.49	99	

Table No.5: Robustness for Levofloxacin

S.No	Injection number	Sample Name	Peak Name	RT	Area	USP Tailing	USP Plate Count
1	1	TEMP1	Levofloxacin	2.603	2345495	1.53	4946
2	2	TEMP2	Levofloxacin	2.399	2353403	1.55	4924
3	3	FLOW1	Levofloxacin	3.190	3142488	1.61	4536
4	4	FLOW2	Levofloxacin	1.924	1788916	1.47	5022

Table No.6: Robustness for Azithromycin

S.No	Injection number	Sample Name	Peak Name	RT	Area	USP Tailing	USP Plate Count	USP Resolution
1	1	TEMP1	Azithromycin	3.815	5449851	1.40	6210	7.32
2	2	TEMP2	Azithromycin	3.615	5478751	1.42	6285	7.38
3	3	FLOW1	Azithromycin	4.816	7313864	1.43	6067	7.20
4	4	FLOW2	Azithromycin	2.897	4258953	1.39	6237	7.38

Table No.7: LOD and LOQ for Azithromycin and Levofloxacin

S.No	Injection number	Sample Name	Peak Name	RT	Area	s/n
1	1	LOD	Levofloxacin	2.399	498857	3.04
2	2	LOQ	Levofloxacin	2.402	250741	9.37
3	3	LOD	Azithromycin	3.606	1306399	3.16
4	4	LOQ	Azithromycin	3.612	2901375	10.44

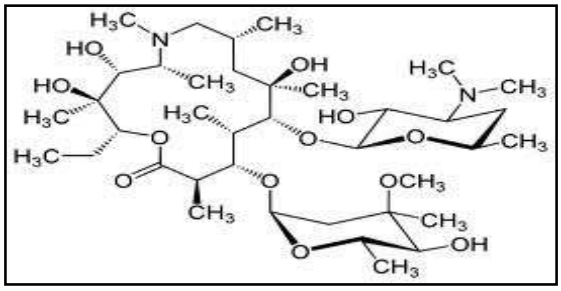


Figure No.1: Structure of Azithromycin

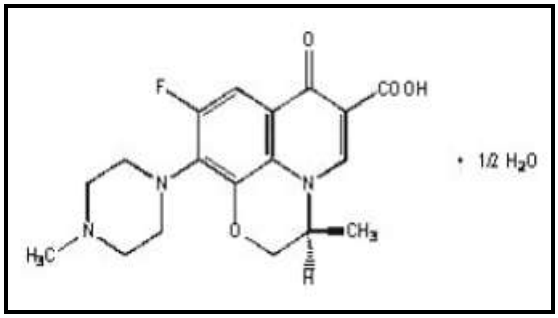


Figure No.2: Structure of Levofloxacin

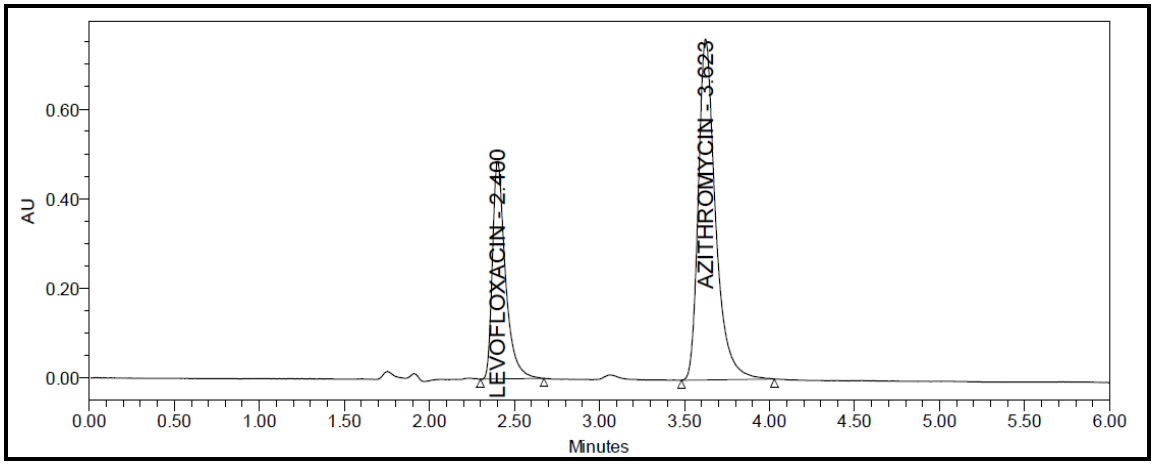


Figure No.3: Standard Chromatogram for Azithromycin and Levofloxacin

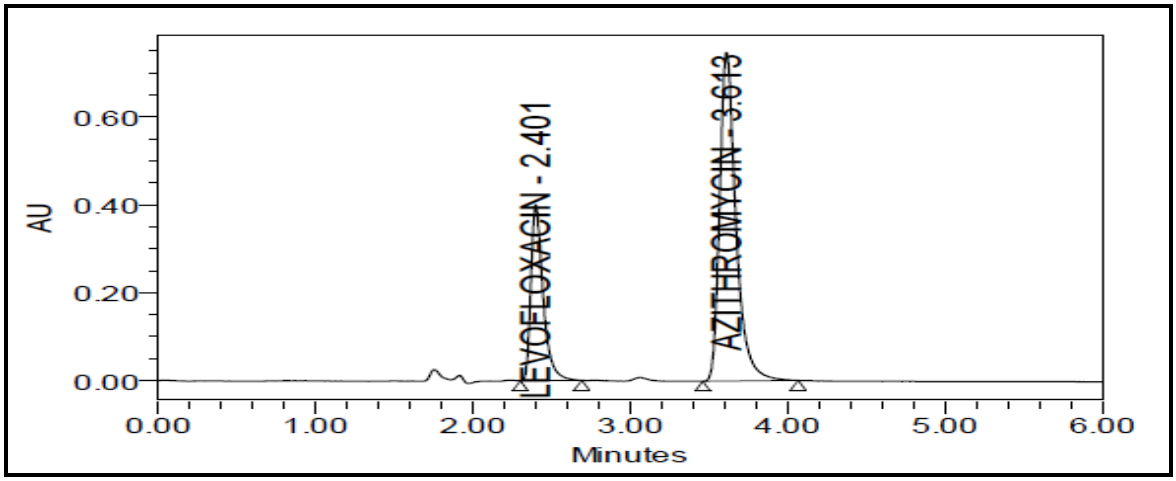


Figure No.4: Formulation Chromatogram for Azithromycin and Levofloxacin

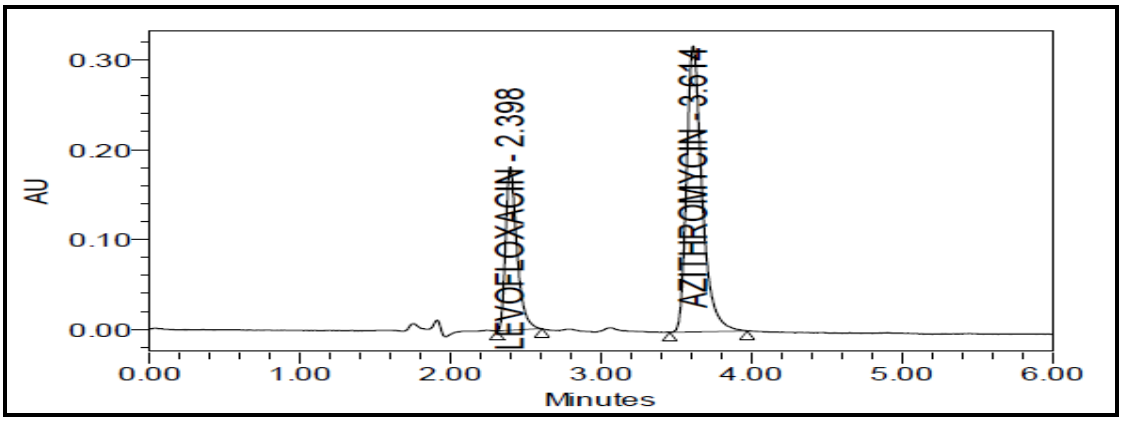


Figure No.5: Accuracy Chromatograms-50% of Azithromycin and Levofloxacin

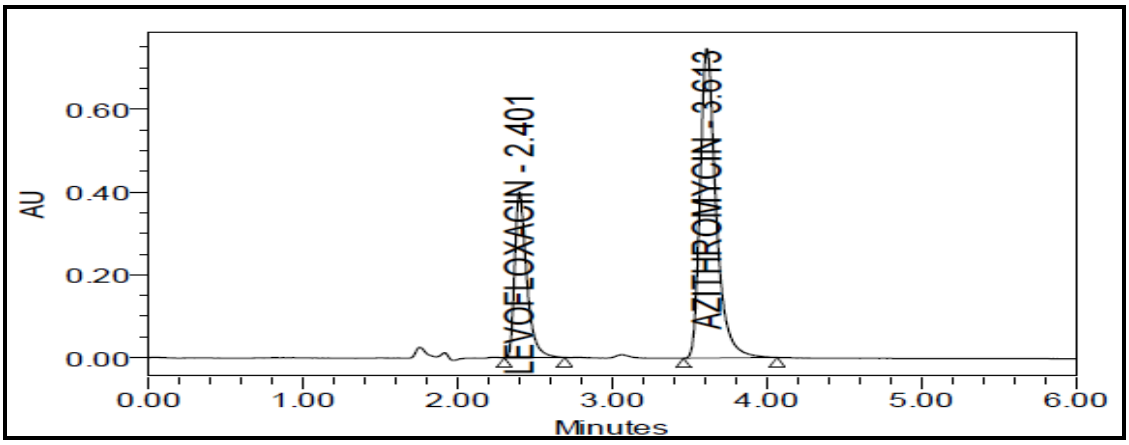


Figure No.6: Accuracy Chromatograms -100% of Azithromycin and Levofloxacin

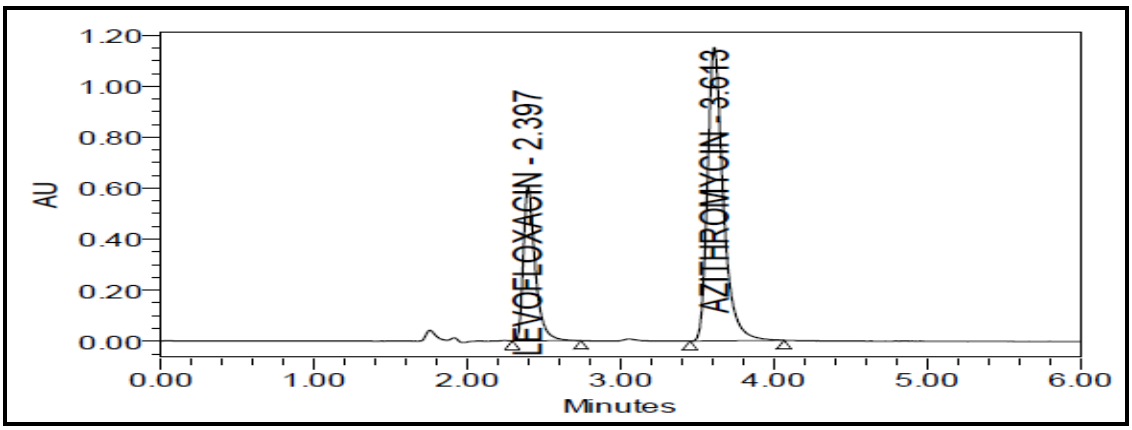


Figure No.7: Accuracy Chromatograms - 150% of Azithromycin and Levofloxacin

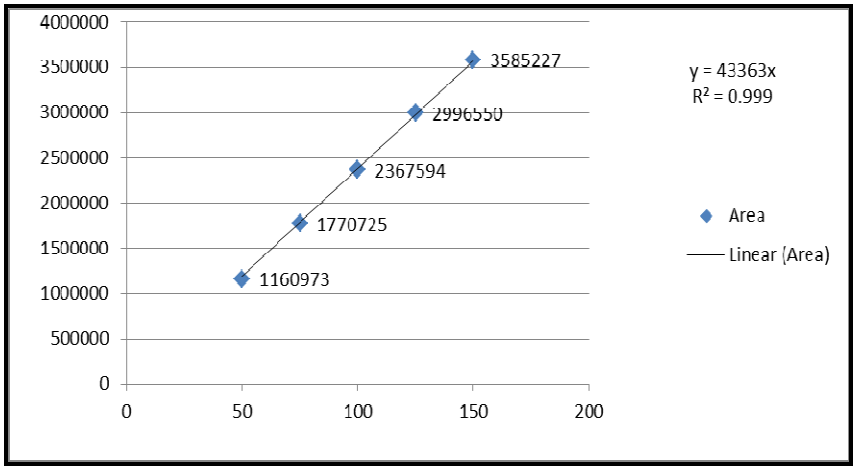


Figure No.8: Linearity Curve for Levofloxacin

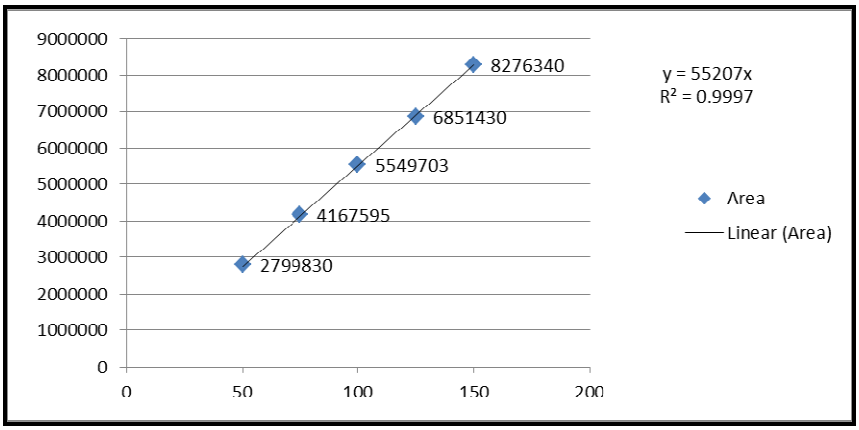
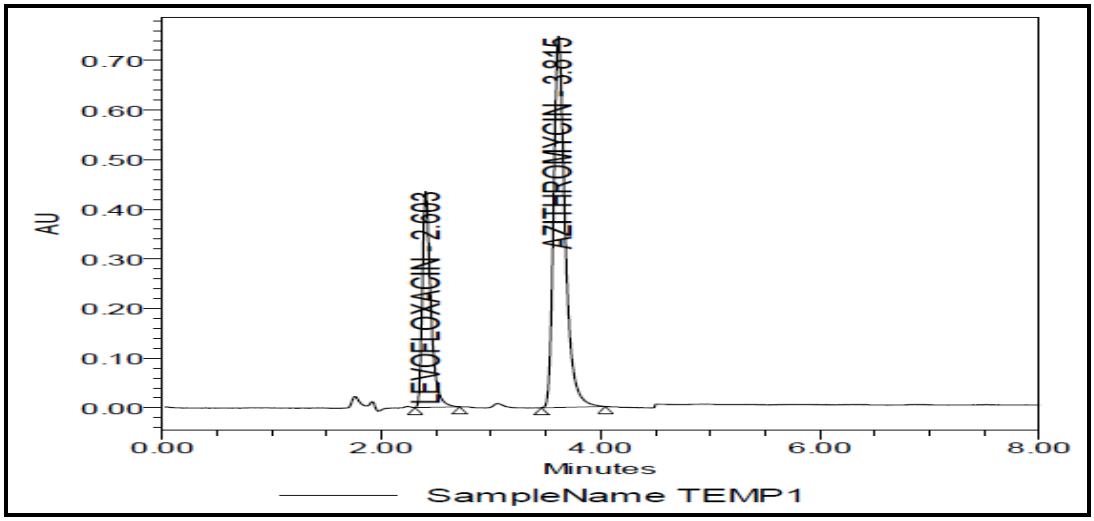
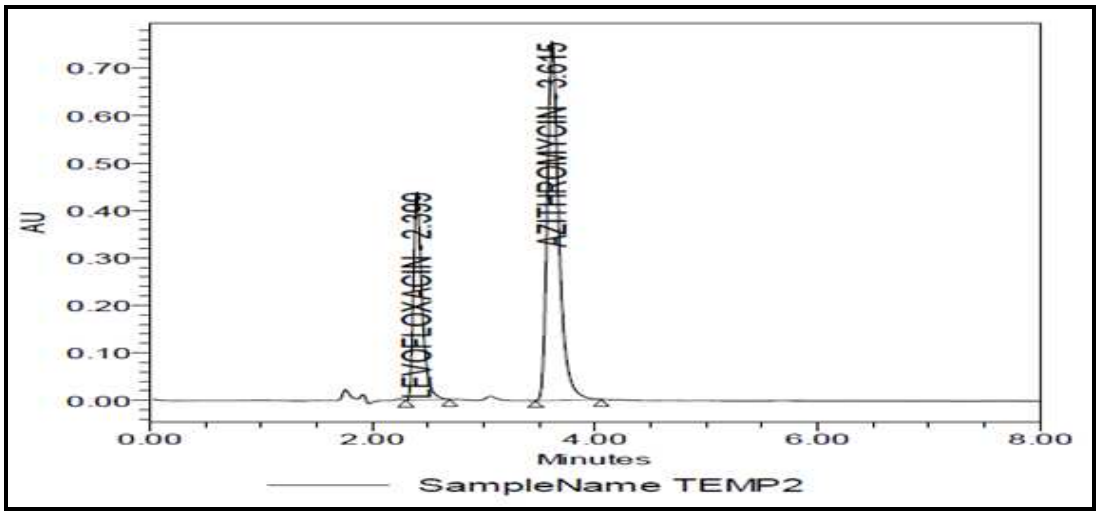
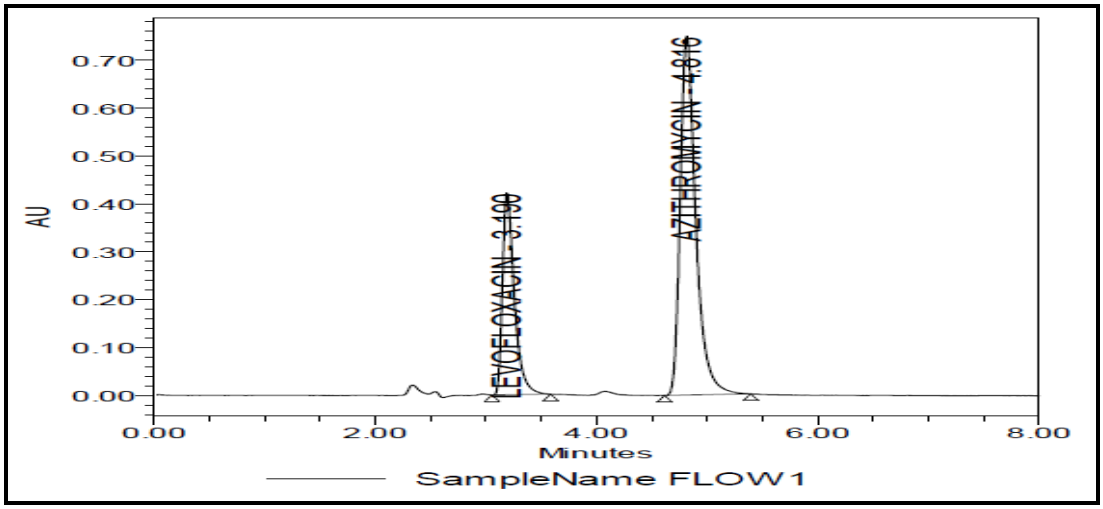


Figure No.9: Linearity for Azithromycin




AU
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
LEVOFLOXACIN - 2.399
AZITHROMYCIN - 3.615
0.00
2.00
4.00
6.00
8.00
Minutes
SampleName TEMP2


AU
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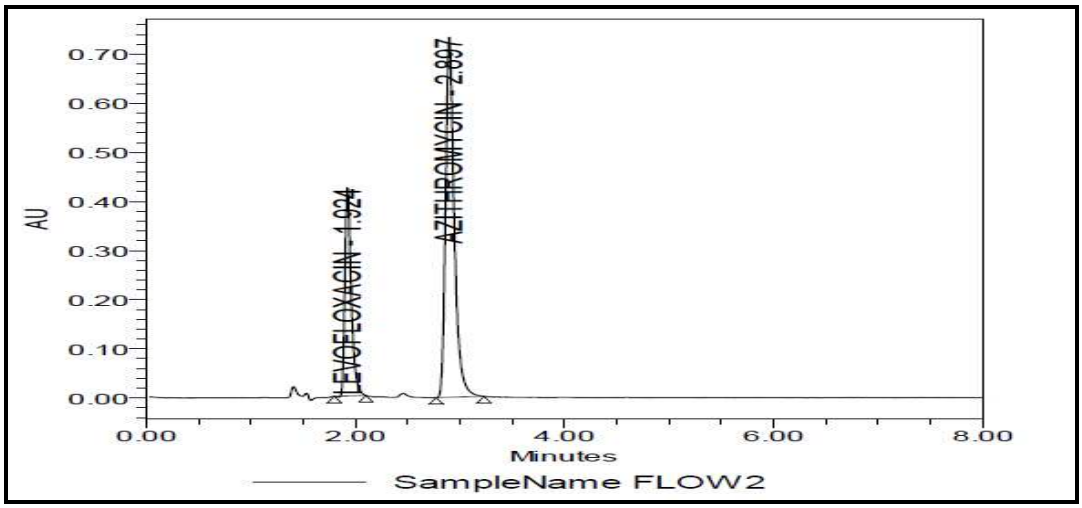
0.60
0.50
0.40
0.30
0.20
0.10
0.00
LEVOFLOXACIN - 3.190
AZITHROMYCIN - 4.816
0.00
2.00
4.00
6.00
8.00
Minutes
SampleName FLOW1


AU
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0.50
0.40
0.30
0.20
0.10
0.00
LEVOFLOXACIN - 1.924
AZITHROMYCIN - 2.897
0.00
2.00
4.00
6.00
8.00
Minutes
SampleName FLOW2

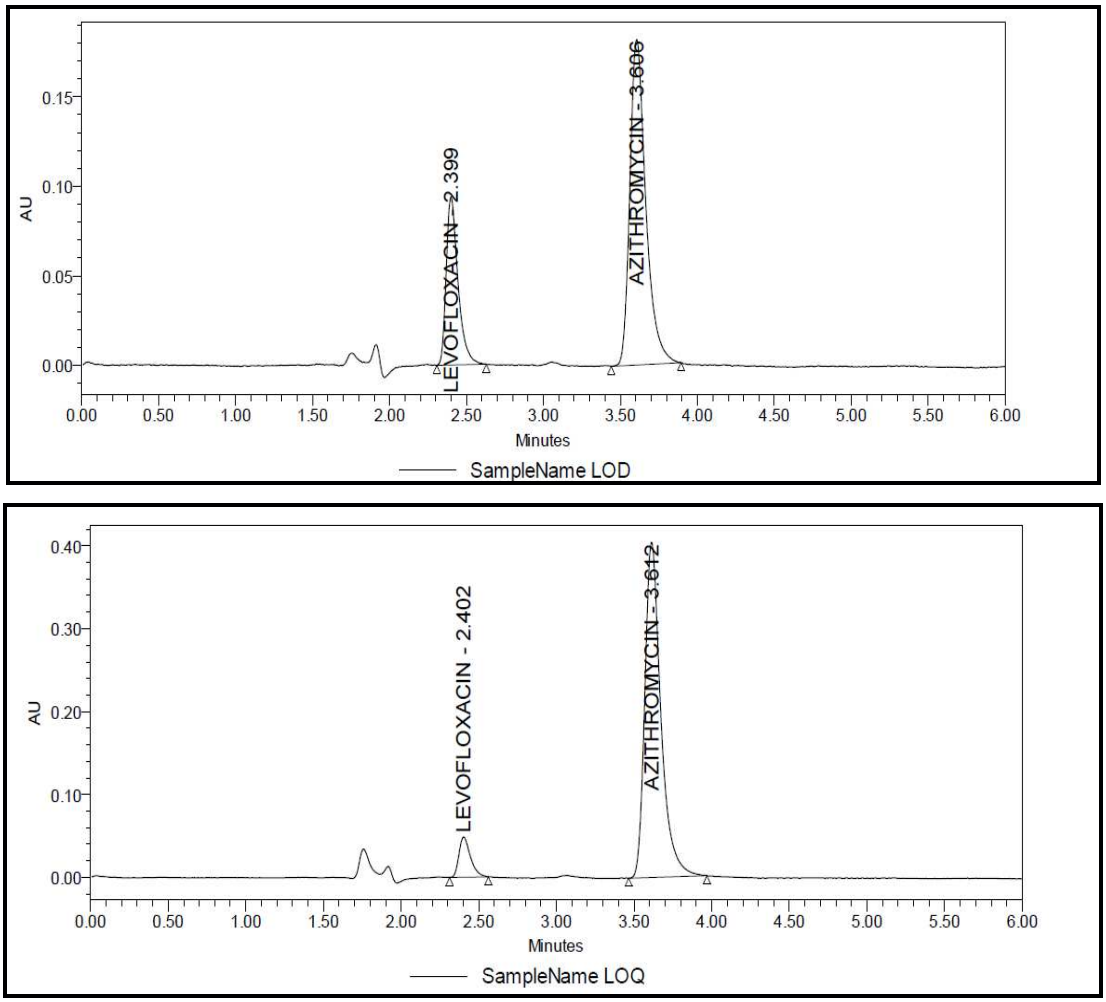

Figure No.10: LOQ and LOD Results

CONCLUSION

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

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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