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Research Article

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS QUANTITATION OF CEFIXIME AND OFLOXACIN IN BULK DRUG AND IN PHARMACEUTICAL FORMULATION

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Abstract: This work is concerned with the simultaneous determination of cefixime and ofloxacin in a bulk drug and pharmaceutical formulations by high performance liquid chromatographic (HPLC) method. Chromatographic separation was achieved on Hypersil BDS column, with Potassium dihydrogen phosphate(PH8.9): acetonitrile (60:40v/v) as mobile phase at flow rate of 1ml/min. Quantification was achieved with PDA detector at 288nm.The retention time for cefixime and Ofloxacin was found to be 4.7 and 6.8mins,respectively. The linearity for both the drugs were observed in the concentration range of 50-150µg/ml with mean accuracies 98-101.The method was successfully applied to pharmaceutical formulation because chromatographic interferences from tablet excipients were found.The method retains its accuracy and precision when the standard addition technique was applied.

Key words: Cefixime, Ofloxacin, High performance thin layer chromatography, validation.

INTRODUCTION

Cefixime is a third generation cephalosporin antibiotic used to treat infections caused by bacteria. The bactericidal action of cephalosporin is due to the inhibition of cell wall synthesis. It binds to one of the penicillin binding proteins (PBPs) which inhibit the final transpeptidation step of the peptidoglycan synthesis in the bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death. Chemically , it is (6R, 7R)-7- { [2-(2-amino -1, 3-thiazole–4-yl)-2-(carboxymethoxyimino)acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo- [4.2.0]oct-2-ene-2-carboxylic acid with molecular formula is $C_{16}H_{15}N_5O_7S_2$, molecular weight is 453.452 g/mol.[1-2]

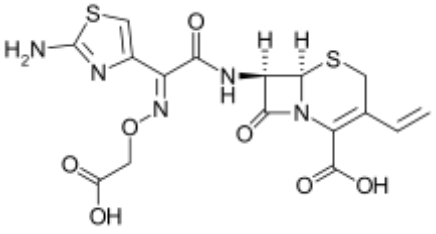


Fig: Cefixime

Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. Ofloxacin was developed as a broader-spectrum analog of norfloxacin. IUPAC name of ofloxacin is 9-Fluro-2-3 dihydro-3-methyl-10- (4-methyl 1-piperazinyl) - 7-oxo-7H- pyrido [1, 2, 3-de] 1, 4 benzoxazine-6-carboxylic and molecular formula is $C_{18}H_{20}FN_3O_4$ and molecular weight of ofloxacin is 361.368 g/mol. Ofloxacin is used to treat certain infections including bronchitis, pneumonia, and infections of the skin, bladder, urinary tract, reproductive organs, and prostate . It works by killing bacteria that cause infections. Antibiotics will not work for colds, flu, or other viral infections.

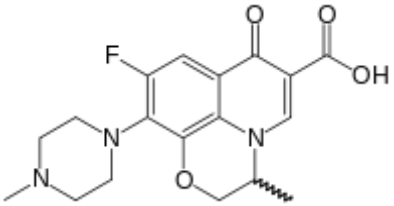


Fig: Ofloxacin

Literature survey revealed that a number of methods are available for estimation of single drugs like spectrophotometric, HPLC, HPTLC etc. The combination of these two drugs are not official and there are a few methods available for quantitative estimation of cefixime and ofloxacin in combination or with other drug combination in pharmaceutical dosage form as well as biological fluids like HPTLC[5], Spectroscopic [6-7] and RP-HPLC[8-9].

Literature survey does not reveal any simple RP-HPLC or other method for

simultaneous estimation of CEFI and OFLO in combined dosage forms. The present communication describes simple, sensitive, accurate and precise RP-HPLC method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIAL AND METHODS

Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, and Waters Empower2 software and thermo hypersil BDS column (250mmx4.6mm, particle size 5µm).

Chemicals and Reagents

Cefixime and ofloxacin was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai. and milli Q water.

HPLC conditions

The mobile phase consisting of potassium di hydrogen phosphate (pH 8.9 adjusted with orthophosphoric acid) and 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 detection was monitored at 288nm and the run time was 8min. The volume of injection loop was 10µl 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:

Cefixime: An accurately weighed quantity, 40 mg of cefixime was transferred into 100ml of volumetric flask and add 30ml of water and sonicate for 15 mins make up the volume with water.

Ofloxacin: An accurately weighed quantity, 40mg of saxagliptin was transferred into 100ml ofvolumetric flask and add 30ml of water and sonicate for 15mins make up the volume with water.

Preparation of sample preparation:

An accurately weigh 8 tablets and calculate average weight of those tablets and crushed.transfer the tablet powder weigh about 324mg of sample into 50ml of volumetric flask add 15ml of water and sonicate for 30mins and filter through the 0.45µm filter paper and make up the volume with water.Transfer above solution 5ml into 25ml volumetric flask and make up the volume.

METHOD VALIDATION

Specificity

The specificity was established by preparing a cefixime and ofloxacin standard at 0.5% level of test concentration and injected 6 times into HPLC system as per the test procedure.

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.

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. From the data obtained, the developed RP-HPLC method was found to be precise.

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 $y = 25655x + 35739$ (R^2=0.999) for cefixime and $y = 32696x + 6933.8$ (R^2=0.9992) for ofloxacin. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The calibration curves are shown in Fig. 3 and Fig 4 respectively.

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 RPHPLC method developed, are rugged and robust.

System suitability studies :

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. 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**.**

Limit of detection(LOD) and Limit of quantification(LOQ):

LOD and LOQ were calculated for the sensitivity of the method they were qualified based on the signal to noise ratio. LOD is lowest detectable concentration of analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines

LOD= 3.3× SD/slope

LOQ= 10×SD/slope

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 cefixime and ofloxacin 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 method accuracy was evaluated by recovery studies . cefixime and ofloxacin recovery was founded 98-102% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate(table3&4) . linearity calibration curve was given below fig:3&4 and plot the graph three different concentration versus areas to construct the linear regression equation and to calculate the value of corelation co efficient. Linear correlation was found to be Y = 25655x + 35739 for cefixime and y = 32696x + 6933.8 for ofloxacin(fig3%4) . precision results were shown by table 2. The intra day and inter day variations was caluculated in terms of %RSD and results was found to be intra day and inter day respectively. Method robustness results was given by table5&6. Stability studies are given in table 7&8.

CONCLUSION

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

Table1: System suitability parameters

Parameters	cefixime	ofloxacin
Correlation coefficient	0.999	0.9992
Regression equation	y = 25655x + 35739	y = 32696x + 6933.8
LOD	0.010608	0.011061
LOQ	0.03589	0.03687
Theoritical plates	7091	8023
Tailing	1.094	1.023

Table2 Precision for cefixime and ofloxacin

S NO	Sample weight	Area(cef)	Area(oflo)	%Assay(cefi)	Assay(oflo)
1	324	2672453	3390135	98	99
2	324	2658925	3377272	98	98
3	324	2687604	3373975	99	98
4	324	2651358	3359122	99	98
5	324	2650379	3345891	98	97
6	324	2657580	3334463	98	97

Table3 Accuracy for cefixime

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	%recovery	mean
50%	155	1301868	189.444	191.72	101	101
50%	155	1299018	189.444	191.30	101	
50%	155	1309120	189.444	192.79	102	
50%	155	1287084	189.444	189.55	100	
50%	155	1278315	189.444	188.25	99	
50%	155	1291447	189.444	190.19	100	
100%	324	2703657	396.000	398.16	101	101
100%	324	2702194	396.000	397.94	100	
100%	324	2701857	396.00	397.90	100	
150%	470	3910729	574.444	575.92	100	100
150%	470	3936009	574.444	579.65	101	
150%	470	3862946	574.444	586.89	99	
150%	470	3884864	574.444	572.11	100	
150%	470	3921177	574.444	577.46	101	
150%	470	3892331	574.444	573.21	100	

Table4 Accuracy for Ofloxacin

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	%recovery	mean
50%	155.00	1648699	189.444	191.85	101	101
50%	155.00	1640830	189.444	190.94	101	
50%	155.00	1647048	189.444	191.66	101	
50%	155.00	1625939	189.444	191.21	100	
50%	155.00	1648699	189.444	191.85	101	
50%	155.00	1645487	189.444	191.48	101	
100%	324.0	3386614	396.000	394.09	100	99
100%	324.0	3382455	396.000	393.61	99	
100%	324.0	3386620	396.00	394.09	100	
150%	470.00	4844748	574.444	563.77	98	98
150%	470.00	4864897	574.444	566.11	99	
150%	470.00	4777646	574.444	555.96	97	
150%	470.00	4808880	574.444	559.60	97	
150%	470.00	4866179	574.444	566.26	99	
150%	470.00	4829363	574.444	561.98	98	

Table6 Robustness for cefixime:

S No	Sample name	Change	Name	RT	Area	Tailing	Platecount
1	Flow1	1.2ml/min	Cefixime	5.953	3299200	1.115	7629
2	Flow2	0.8ml/min	Cefixime	4.009	2179531	1.076	5756
3	Temp1	50^{o}C	Cefixime	4.842	2645834	1.081	6864
4	Temp2	40^{o}C	cefixime	4.849	2630312	1.070	6824

Table6 Robustness for ofloxacin:

S No	Sample name	Change	Name	RT	Area	Tailing	Platecount
1	Flow1	1.2ml/min	Ofloxacin	8.441	4120154	1.036	8643
2	Flow2	0.8ml/min	Ofloxacin	5.745	2703853	1.008	6589
3	Temp1	50°C	Ofloxacin	7.047	3287065	1.016	7655
4	Temp2	40°C	ofloxacin	6.810	3283377	0.993	9818c

Fig1:standard chromatogram

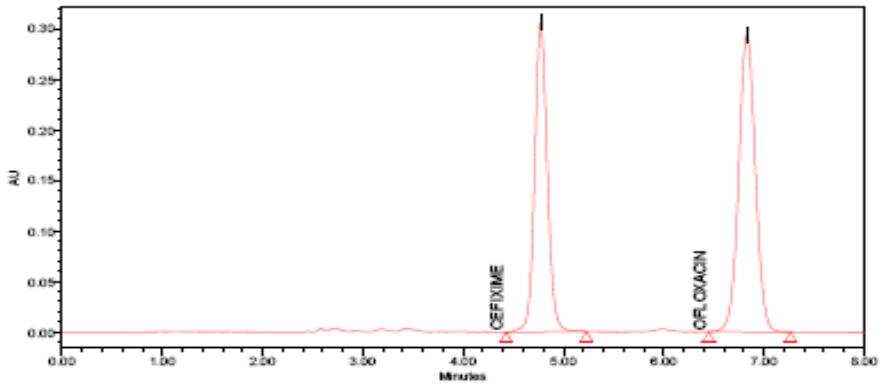


Fig2:chromatogram for formulation:

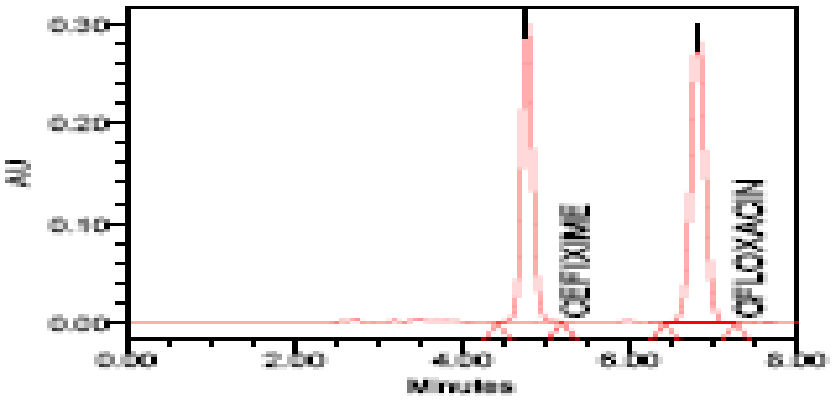


Fig:3 linearity curve for cefixime:

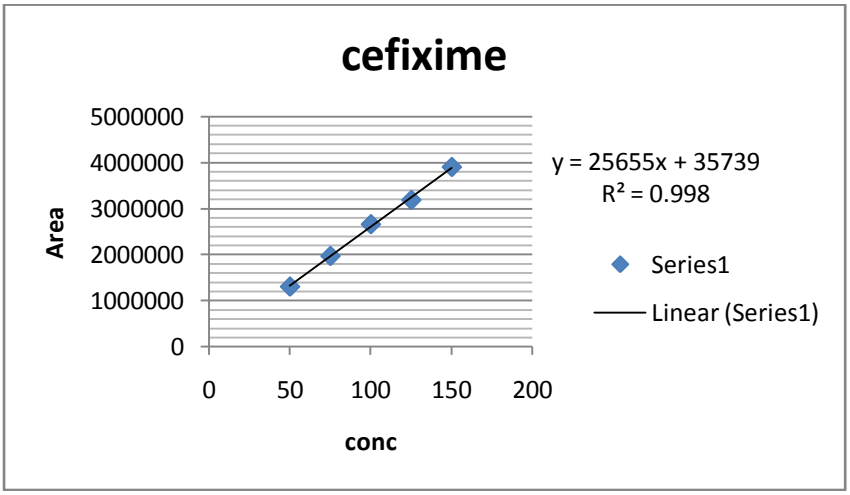
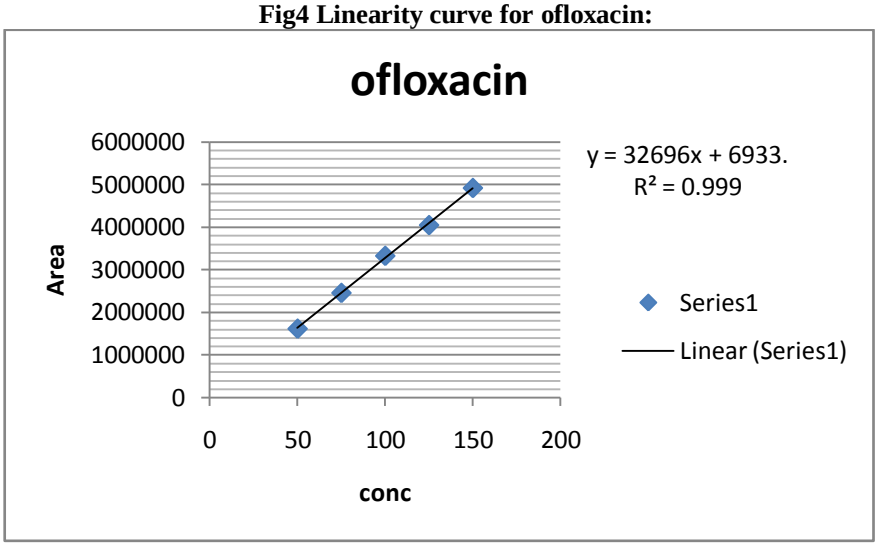


Fig4 Linearity curve for ofloxacin:



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