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Original Article

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME AND MOXIFLOXACIN IN COMBINED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop a simple, selective and rapid reversed phase high performance liquid chromatographic (HPLC) method for the analysis of cefixime and moxifloxacin in combined pharmaceutical dosage form as per ICH guidelines.

Methods: The separation was achieved from C_{18} column at 35^{0}C with a mobile phase consisting of methanol: 0.05M heptane sulfonic acid sodium salt,0.5 ml THF and 0.5 ml TEA [75: 25 v/v]. pH-3.8 was adjusted with ortho phosphoric acid at a flow rate of 0.4 ml/min and the retention time was about 6.08 minutes for cefixime and 6.94 minutes for moxifloxacin. The method was selective to cefixime and moxifloxacin able to resolve the drug peak from formulation excipients.

Results: The calibration curve was linear over the concentration range of 20-120 µg/ml (r^2 = 0.999) for both drugs. The proposed method was found to be accurate and precise and linear within the desired range. The limit of detection (LOD) and limit of quantitation (LOQ)were calculated statically. Recoveries do not differ significantly from 100% which show there was no interference from the common excipient used in tablet formulation indicating accuracy and reliability of the method. The method was validated as per ICH guidelines and found to be accurate, precise and rugged. The method was validated in terms of linearity, accuracy, precision, specificity, LOD and LOQ.

Conclusion: A novel, simple, selective and rapid reversed phase high performance liquid chromatographic (HPLC) method was developed for the analysis of cefixime and moxifloxacin in tablets. Hence,the method can be used for the routine analysis in various pharmaceutical industries.

Keywords: Simultaneous estimation, Cefixime, Moxifloxacin, RP- HPLC, Validation, Tetrahydrofuran (THF), Triethylamine(TEA).

INTRODUCTION

Cefixime (CEF) (Figure 1) is official in United State Pharmacopoeia[1],British Pharmacopoeia[2]. Cefixime is (6R,7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2- (carboxy methoxyimino) acetyl]amino} -3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid. It is anantibacterial agent and used to treat urinary tract infections,otitis[3] bronchitis, pneumonia,Prostatitis, syphilis and infections of reproductive organs[4, 5, 6]. Moxifloxacin is official in British Pharmacopoeia[7]. Moxifloxacin(MOX)(Figure 2)is a 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS, 7aS) -octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3 carboxylic acid hydrochloride[7]. It is afourth generation fluoroquinolone broad spectrum antibiotic agent used in conjunctivitis[8]. Literature survey reveals that number of methods such as spectrophotometric[4, 9,10], HPLC[11, 12], HPTLC[13],colorimetric[14], spectroflurometric[15]are reported for the estimation of CEF from its formulation or biological fluids. Similarly number of methods such as spectrophotometric[16, 17] spectrofluorometric[18], RP-HPLC[19, 20], voltametric[21] were reported for the estimation of moxifloxacinfrom its formulation or biologicalfluids.

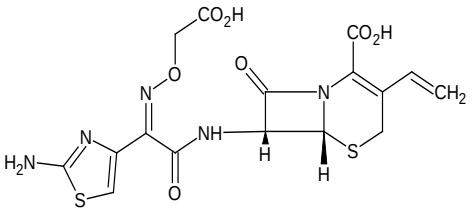

Fig. 1: Structure of cefixime

This paper is in continuation with our work [24-29], where we studied chromatographic method for single or multicomponent drugs. So, present study was aimed to develop andvalidate RP-HPLC method forsimultaneous estimation of cefixime andmoxifloxacin from combined dosage form which would be simple, cost effective and easily adopted by small laboratories. The proposed method was validated according to ICH guidelines.[22, 23].

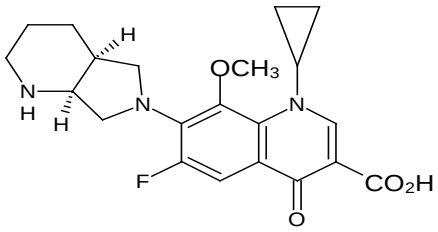

Fig. 2: Structure of moxifloxacin

MATERIALS AND METHODS

Apparatus

The liquid chromatographic system of Perkin Elmer series 200 (Mumbai, India) containing quaternary gradient pump, variable wavelength programmable PDA detector, and auto sampler with 10 μL fixed loop was used. For analysis a hypersil C_{18} column with 250 ×4.6 mm i. d. and 5 μm particle sizes were used as stationary phase.

Reagents and materials

Working standard of cefixime was pursued as a gift sample from FDC Pharma Ltd., (Mumbai, India) and moxifloxacinwas pursued as a gift sample from Dr. Reddy's lab Ltd., (Hydrabad, India). All chemicals and solvents of HPLC grade and were purchased from Rankem Ltd., (Mumbai, India). Marketed formulation of tablet containing cefixime and moxifloxacin was used as the sample which was purchased from the local market.

Preparation of mobile phase

The mobile phase was prepared by mixing methanol: 0.05M heptanes sulfonic acid sodium salt, 0.5 ml THF and 0.5 ml TEA in 75:25 v/v ratio and pH was adjusted to 3.8 with orthophosphoric acid. The whole mobile phase was sonicated for 15 min and then filtered through 0.45 µm membrane filter paper.

Standard stock solution of Cefixime and Moxifloxacin (400 µg/ml)

40 mg of standard drugs cefixime (CEF) and moxifloxacin (MOX) was dissolved separately in 100 ml of the mobile phase. Then the volumes were made up to the mark with mobile phase to get 400 µg/ml of standard stock solutions and sonicated for 10 minute. These stock solutions were filtered through 0.45 µm membrane filter paper. For the preparation of working standard, suitable aliquots of stock solutions were pipette out and volumes were made up to the mark with mobile phase.

Chromatographic conditions

The hypersil C_{18} column (5 μ × 250 mm × 4.6 mm) equilibrated with mobile phase methanol: 0.05 M heptanes sulfonic acid sodium salt, 0.5 ml THF and 0.5 ml TEA (75:25 v/v), pH 3.8 adjusted with orthophosphoric acid was used. The flow rate was maintained at 0.4 mL/min. Eluents were monitored with PDA detector at 284 nm,temperature was maintained at 35 °C and the injection volume was 10 μL. Total run time was kept for 10 min.

Preparation of calibration curve of cefixime and moxifloxacin

Appropriate aliquots of the standard stock solution of CEF and MOX were pippetted out and transferred to a series of 10 ml volumetric flasks to obtain a concentration range of 20-120 µg/ml for both the drugs. Triplicate dilutions of each concentration were prepared separately and 10 µl of each concentration of the drug were injected into the HPLC system two times separately.

The chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were determined and a standard calibration curve of peak area against concentration was plotted. The individual chromatograms of standard CEF and MOX are shown in (**Fig. 3, 4**). Observation for calibration curve are shown in table 1.

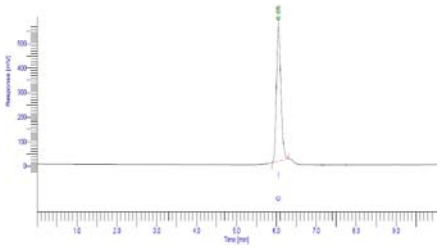

Fig. 3: Chromatogram of CEF

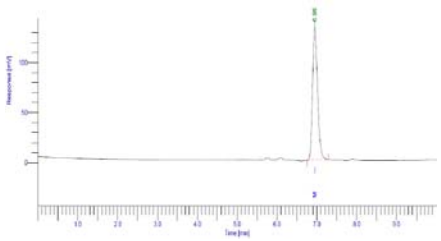

Fig. 4: Chromatogram of MOX

Analysis of the marketed formulation

Twenty tablets were weighed accurately and triturated to produce fine powder. A quantity equivalent to 400 mg of CEF and 400 mg of MOX was weighed and transferred to 100 ml volumetric flask and volume was made up to 100 ml with mobile phase. The contents were sonicated for 10 min and filtered through 0.45 µm membrane filter paper.

Table 1: Observation for standard calibration curve of CEF & MOX

S. No.	Conc. (µg/ml)	Peak area CEF	Peak area MOX
1	20	1106091	229578
2	40	2155165	442897
3	60	3226470	669104
4	80	4324397	907328
5	100	5422408	1114156
6	120	6411023	1344018

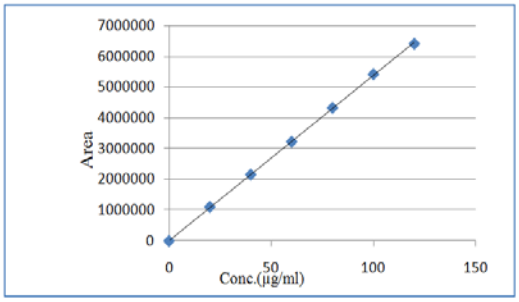

Fig. 5: Calibration curve for CEF

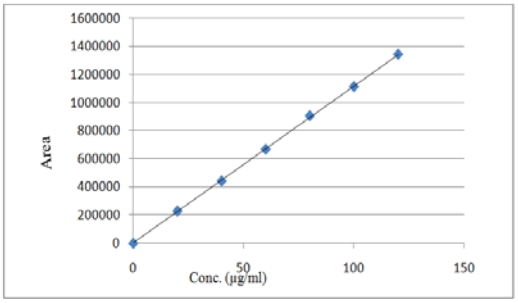

Fig. 6: Calibration curve for MOX

By appropriate dilution of this stock solution with mobile phase, further sample solutions were prepared within the concentration range for two drugs. A 10 µl volume of each sample solution was injected into HPLC system for six times under the chromatographic condition as stated above. Result of analysis of the marketed formulation presented in Table 2.

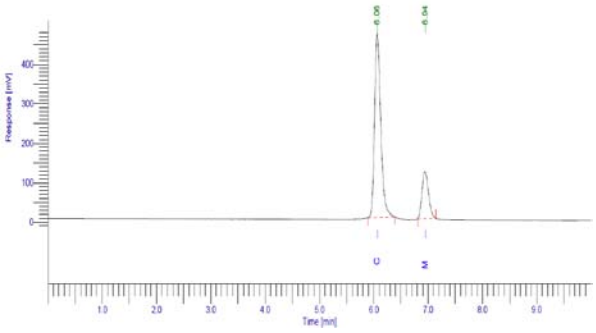

Fig. 7: Chromatogram for mixture of CEF and MOX

Method validation

Linearity

The calibration curves were found to be linear over concentration range 20-120 µg/ml for both CEF and MOX. The results of linearity studies are given in Table 3.

Recovery study

Recovery study was carried out at three levels i. e. 80, 100 and 120 % of the label claim of the tablet formulation as per ICH guidelines. Results of recovery study are given in Table 5.

Precision

Precision of the method was verified by using stock solutions in concentration containing 80 µg/ml of CEF and MOX. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Interday precision was carried out by performing the assay of six sample sets after 24 hrs and 48 hrs. The results of precision study are given in Table 6.

Robustness

To evaluate the robustness, the developed method was subjected to small deliberate variations in the optimized method parameters like the variation of flow rate ±0.2 ml/min, temperature of column ±2^{0}C, ratio of mobile phase ±2%, pH of mobile phase±0.2. The method was found to be robust as the slight deliberate variations in parameters did not lead to changes in retention times of peak of interest. While evaluating the robustness data, it was observed that systemsuitability parameters (e. g. tailing factor, plate counts, resolutions etc.) were found to be within the specified limits under those deliberately varied conditions, which ensures that the validity of the analytical procedure was maintained whenever used. The result of robustness study is summarized below in Table 7, 8, 9, 10.

Limit of detection and limit of quantitation (LOD and LOQ)

The sensitivity of measurement for CEF and MOX was estimated in terms of the limit of quantitation (LOQ). The smallest amounts detected under the chromatographic conditions used were estimated in terms of the limit of detection (LOD). LOQ and LOD were calculated by using following equations.

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the peak areas of the drugs, taken as a measure of noise, and S is the slope of the corresponding calibration curve.

RESULTS AND DISCUSSION

The proposed RP-HPLC method, allows a rapid and accurate quantitation of cefixime and moxifloxacin in combined pharmaceutical dosage form as per ICH guidelines. The chromatograms of standard CEF and MOX are shown in (Figure 3, 4). The standard calibration curves (Figure 5, 6) were plotted separately as peak area against the respective concentration of CEF and MOX. The linearity of both drugs was found in acceptable range. The accuracy of the proposed method was determined by recovery studies (Table 5), indicating an agreement between the true value and found value. Precision was calculated as interday and intraday variation of given drugs. Percent relative standard deviations for estimation of cefixime and moxifloxacin under intraday and interday variations were found to be less than 2 (Table 6). For robustness studies in all deliberately varied conditions percent relative standard deviations were found to be less than 2 % (Table 7 - 10).

Table 2: Results of marketed tablet formulation

Formulation	*Mean Recovery	SD	%RSD
CEF	99.98	0.320	0.323
MOX	99.92	0.050	0.0501

*Denotes average of 6 determinations

Table 3: Linear regression data for calibration curve of CEF and MOX

Drug	Linearity range (µg/ml)	r^2	Slope	Intercept
CEF	20-120	0.999	53634	17051
MOX	20-120	0.999	11189	1122

Table 4: Results of LOD & LOQ

Parameters	CEF	MOX
LOD (µg/ml)	0.00061	0.00294
LOQ (µg/ml)	0.002019	0.009760

Table 5: Results of Recovery Study

Level of	% Recovery*		SD		% RSD	
recovery	CEF	MOX	CEF	MOX	CEF	MOX
80%	99.87	99.87	0.0467	0.0383	0.0468	0.03835
100%	99.96	99.94	0.0608	0.0526	0.0608	0.05263
120%	99.95	99.89	0.0079	0.0375	0.0079	0.03754

Table 6: Results of Precision

Formulation	Parameter	Intra-day precision*	Inter-day precision*
CEF	% Mean	99.82	99.87
	SD	0.39	0.35
	%RSD	0.3907	0.3504
MOX	% Mean	99.89	99.84
	SD	0.058	0.059
	%RSD	0.0580	0.0590

Table 7: Flow Rate (ml/min)

Flow	Retention Time (min)		Tailing Factor		Peak Area		% Content Found	
	CEF	MOX	CEF	MOX	CEF	MOX	CEF	MOX
0.2	12.35	14.73	1.41	1.33	3520029	759106.2	99.98	99.93
0.4	6.06	6.94	1.361	1.244	3520483	759626.5	99.99	100
0.6	4.02	4.59	1.34	1.19	3499705	760038.2	99.40	100.05
***Mean**	7.476	8.753	1.370	1.254	3513405	759590.3	99.79	99.99
±S. D.	±4.34	±5.30	±0.035	±0.0706	±11867.	±467.03	±0.337	±0.06

Table 8: Column temperature

Temp.	Retantion Time		Tailing Factor		Peak Area		% Content Found	
	CEF	MOX	CEF	MOX	CEF	MOX	CEF	MOX
33	6.04	6.9	1.43	1.32	3518692	757273.5	99.94	99.69
35	6.06	6.94	1.36	1.25	3498490	759697	99.37	100.00
37	6.01	6.84	1.31	1.21	3496020	755977.2	99.30	99.51
***Mean**	6.036	6.893	1.366	1.26	3504401	757649.2	99.54	99.73
±S. D.	±0.025	±0.050	±0.06	±0.055	±12438.3	±1888.15	±0.3533	±0.248

Table 9: pH of mobile phase

pH	Retantion Time (min)		Tailing Factor		Peak Area		% Content Found	
	CEF	MOX	CEF	MOX	CEF	MOX	CEF	MOX
3.6	6.14	6.97	1.36	1.41	3500583	753646	99.43	99.21
3.8	6.06	6.94	1.35	1.23	3528133	758829	100.21	99.89
4	6.12	7.38	1.32	1.21	3501232	749989	99.45	98.73
***Mean**	6.10666	7.0966	1.346	1.286	350998	754154.9	99.70	99.27
± S. D	±0.0416	±0.248	±0.0225	±0.1080	±15721.	±4442.25	±0.4465	±0.584

Table 10: Ratio of mobile phase

Ratio	Retention Time (min)		Tailing Factor		Peak Area		% Content Found	
	CEF	MOX	CEF	MOX	CEF	MOX	CEF	MOX
27:73	6.12	7.47	1.4	1.43	3550302	752911.2	100.84	99.11
25:75	6.06	6.94	1.35	1.23	3510483	759926.5	99.71	100.03
23:77	6.06	7.09	1.32	1.19	3501884	750200.6	99.47	98.75
***Mean ±S. D.**	6.08	7.166667	1.359	1.286	3520890	754346.1	100.01	99.30
	±0.0346	±0.2731	±0.0400	±0.1268	±25832	±5019.19	±0.73	±0.646

CONCLUSION

The objective of the work was to develop the simple, accurate, precise and sensitive HPLC method for the estimation of cefixime and moxifloxacin in bulk and multicomponent formulation. From the results obtained by all parameters, it is concluded that developed RP-HPLC method is suitable for the simultaneous estimation of cefixime and moxifloxacin in bulk and multicomponent formulation. The concentration of CEF and MOX in pharmaceutical dosage form could be satisfactorily determined using isocratic RP-HPLC system with PDA detector. This method has been found suitable for the routine analysis of pharmaceutical dosage forms in QC and R & D laboratories for products of similar type and composition.

CONFLICT OF INTERESTS

Declared None.

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