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Simultaneous Estimation of *Cefixime* and *Moxifloxacin* in Bulk and its Pharmaceutical Dosage form by RP-HPLC

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ABSTRACT

A simple, efficient and reproducible reverse phase high performance liquid chromatographic method was developed and validated for the Simultaneous determination of cefixime (CEF) and moxifloxacin (MOX) in combined dosage form. Chromatographicseparationofthetwo drugswasperformedonaPurospherBDSC18 column(250mm×4.6mmid, 5µm particlesize). The mobile phase comprising of acetonitrile and 0.01M KH_2PO_4 in a ratio of 40:60 v/v at a flow rate of 1.0ml/min. The detection was made at 276 nm. The retention time of cefixime and moxifloxacin was found to be 3.140± 0.007min and 7.007± 0.006min. Calibration curve was linear over the concentration range of 20-120 µg/ml for both cefixime and moxifloxacin .All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicate the validity of the method. The developed method is also found to be precise, accurate, specific, robust and rapid for the simultaneous determination of cefixime and moxifloxacin in tablet dosage forms.

Key words: Cefixime, Moxifloxacin, Method development and validation, RP-HPLC.

INTRODUCTION

Cefixime trihydrate (CEF) is an orally active third generation semi synthetic cephalosporin. Chemically, CEF is (6R,7R)- [(Z)-2-(2-aminothiazol-4-yl) -2-[(carboxymethoxy) imino] acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0]pet-2-ene-2-carboxylic acid trihydrate, [Figure1.a] CEF is official in USP,[1] BP,[2] and EP.[3]Moxifloxacin (MOX) (1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7- [(4aS,7aS)-octahydro-6H-pyrrolo-[3,4-b]pyridin-6-yl]-4-oxo-3-quinoline carboxylic acid hydrochloride) ,[Figure1.b] is new, fourth generation fluoroquinolone with broaden spectrum of antibacterial activity[4-6]. Moxifloxacin in combination with the 3rd generation cephalosporine cefixime shows great additive and Synergistic effect to treat infectious diseases caused by a Gram-positive or Gram-negative pathogen like *Streptococcus pneumoniae, Pseudomonas*

aeruginosa and *Staphylococcus aureus*[7-8]. The Innovative tablet combination of Cefixime trihydrate and moxifloxacin was recently approved by DCGI and CDSCO. The present investigation carried out on tablet mixture prepared from commercially available individual tablet dosage form of above mentioned drugs. Literature survey shows that various analytical methods have been reported for estimation of cefixime[9-12] and moxifloxacin[13-17] individually and combination with other drugs[18-21]. Only one UV-Visible Spectrophotometric method[22] was reported for its simultaneous estimation. HoweverthereisnoRP-HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. In this communication, a simple, precise, reproducibleand accuratereverse-phase high performance liquid chromatographic method to estimate cefixime and moxifloxacin in tablet dosage for misreported.

MATERIAL AND METHODS

Chemicaland Reagents

The gratis samples of Cefixime (CEF)and Moxifloxacin (MOX) were obtained from Alembic limited, Vadodara and Torrent Pharmaceutical Industries Ltd., Ahmedabad. HPLC grade water and acetonitrile were purchased from E.Merck (India) Ltd., Mumbai. Potassiumdi hydrogen phosphate and orthophosphoric acid of ARGrade were obtained from S.D. Fine Chemicals Ltd., Mumbai.The marketed tablet formulations Suprax®having 400 mg of CEF from Lupin, Mumbaiand Moxif®having 400mg MOX from TorrentPharmaceuticalIndustriesLtd., Ahmedabad were purchased from the local market.

Chromatographicconditions

Theanalysisofthedrug wascarriedoutona Waters HPLC systeme quipped with a reverse phase PurospherBDSC18 column (250mm ×4.6mmid, 5µm particlesize), 2695 binary pump, a20µl injection loop and a 2487dual absorbace detect or and running on Waters Empower software.

Standard preparation

Standard stock solution

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of acetonitrile and phosphate buffer in the ratio of 40:60v/v to get concentration of 1000µg/ml.

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 20-120µg/ml for both CEF and MOX, respectively.

Validation of the method

The developed method was validated as per ICH guidelines[21] in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and system suitability. The accuracy was expressed in terms of percent recovery of the known amount of the active pharmaceutical ingredient in presence of excipients. The precision (%relative standard deviation, %RSD) was expressed with respect to the intraday and interday variation in the expected drug concentrations. Minor changes in pH of the mobile phase, flow rate, column temperature and detector wavelength were studied to evaluate the robustness of the developed assay method.

RESULTS AND DISCUSSION

Optimization of the method

The goal of this study was to develop a single isocratic phase HPLC method for the simultaneous determination of cefixime trihydrate and moxifloxacin.During optimizing the method some important parameters like pH of the mobile phase , concentration of the acid or buffer solution, percentage and type of the organic modifier, etc.,were tested for a good chromatographic separation. Trials showed that a slightly acidic with reverse phase, aPurospher BDSC18 column gives symmetric and sharp peaks. For this reason,0.01M potassium di hydrogen orthophosphate solution was preferred as an acidic buffer .When triethylamine was used as modifier the method shows a very good resolution between CEF and MOX at pH in the range of six .Finally acetonitrile andpotassium di hydrogen phosphate buffer pH6 with intheratioof40:60 v/v was selected as optimal for obtaining well defined and resolved peaks. The detection of thed rug was monitored at276nm. Theruntime was set at 10min. Under these optimized chromatographic conditions the retention time obtained for the drugscefixime and moxepril

was3.140min and 7.001min, respectively. A typical chromatogram showing the separation of the drug is given in [Figure 2]

Method validation

The developed method was validated as per ICH guidelinesin terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and system suitability.

System suitability test

The system suitability test performed according to ICH guidelines. The observed RSD values at 1% level of analyte concentration were well within the usually accepted values (≤ 2%). Theoretical plates, tailing factor, resolution between CEF and MOX were determined for both assay and dissolution. The results are all within acceptable limits summarized in [Table1].

Specificity

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug

Table 1: System Suitability parameters

S. No	Parameters	Cefixime	Moxifloxacin	Acceptance criteria
1	Retention time	3.12	7.04	
2	RSD of replicate injections	0.115	0.225	Not more than 2%
3	Asymmetric factor	1.16	0.92	Not more than 2
4	Theoretical plates	6178	5210	Not less than 3000
5	Resolution factor		7.41	More than 2

Table 2: Linearity Study

S.No	Parameters	Cefixime	Moxifloxacin
1	Linearity range	20-120µg/mL	50-300 µg/mL
2	Slope	6273.7	8110.4
3	Intercept	6841.1	10790
4	Correlation coefficient(R^2)	0.9996	0.9991
5	Limit of Detection	0.50 µg/mL	0.82 µg/mL
6	Limit of Quantification	2µg/mL	2.5µg/mL

Table 3: Intraday and interday precision data for the quantitative determination of Cefixime and Moxifloxacin

Name of the Drug	Concentration µg/ml	Intraday precision		Interday precision	
		Calculated concentration±SD*	RSD%	Calculated concentation±SD*	RSD%
CEF	40	39.94±0.413	0.41	40.14±0.091	0.54
	60	60.10±0.314	0.22	59.95±0.143	0.22
	80	81.01±0.415	0.81	79.96±0.264	0.71
MOX	40	40.18±0.246	0.42	40.24±0.126	0.62
	60	61.29±0.129	0.49	60.19±0.116	0.12
	80	80.11±0.315	0.41	79.58±0.744	0.84

*Average of six determinations

solution of 20 µg/mL was injected into the column, underoptimized chromatographic conditions, to demonstrate the separationo f both CEF andMOX from any of the impurities, if present. As the rewasno interference of impurities and also no change in there tention time,the method was found to be specificand also confirmed with theresults of analysis of formulation.

Linearity study

The peak areas of CEF and MOX were linear with respect to the concentrations over the range of 20-120µg/mL for both respectively. The slope and intercept value for calibration curve Y =6243.7X—6841.1 (R^2= 0.9996) for CEF and 8110.4X—10790(R^2= 0.9991) for MOX.The results showed that excellent correlation exists between peak area and concentration of the drugs within the concentration range indicated previously. The data was analyzed by "linear regression least squares fit", and the parameters are listed in [Table2].

Limit of detection and Limit of quantification

The linearity for CEF and MOX were performed from 20-120µg/ml. Linearity graph was plotted and the correlation coefficient (R^2) determined. The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD= 3.3× {Residual Standard deviation/Slope}.

The LOD for CEF was confirmed to be 0.5µg/mL and for MOX it was confirmed to be0.82 µg/ml.

The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ= 10× {Residual Standard deviation/Slope}

The LOQ for CEFwas confirmed to be2µg/mL and forMOX it was confirmed to be2.5 µg/ml.

Table 4: Accuracy of the Method

Name of the Drug	Amount (%) of drug added	Theoretical content (µg/ml)	Conc.found (µg/ml)±SD*	Recovery (%)	RE (%)	RSD (%)
CEF	0	40	39.52±0.222	98.8	0.54	0.562
	50	60	59.97±0.325	99.95	0.89	0.541
	100	80	80.65±0.564	100.81	0.11	0.699
	150	100	99.97±0.245	99.97	0.45	0.245
MOX	0	40	40.46±0.354	101.15	0.36	0.874
	50	60	59.95±0.324	99.91	0.76	0.540
	100	80	79.93±0.356	99.92	0.26	0.445
	150	100	100.45±0.698	100.45	0.79	0.694

*SD= standard deviation(n=3),*RSD=SD/Mean×100,

RE(%)=%Relative Error =(Mean assayed concentration-Added Concentration/ Added Conentration×100)

Table 5: Statistical data for Ruggedness

Parameter	Cefixime		Moxifloxacin	
	S.D	%R.S.D	S.D	%R.S.D
Shimadzu and Waters (Different Instrument)	0.312	1.256	0.741	1.245
Day to day	0.211	1.054	0.145	1.547
Analyst to Analyst	0.145	0.984	0.19	1.121

Precision

Intraday and Interday precision were evaluated by determining the corresponding responses three times on the same day and on 3 different days for CEF and MOX(40,60,80 µg/ml).The results of intra- and inter-day variations are shown in [Table3]. The results obtained from intermediate precision also indicated a good method precision. All the data were within the acceptance criteria.

Accuracy

The accuracy of the method was determined by recovery experiments. It was confirmed by studying the recovery at three different concentrations, 50%, 100%,and 150% of those expected by spiking a previously analyzed test solution with additional drug standardsolutions, the analysis being done inreplicate. The %RSD and %relative error in allcases were within the acceptable limit (≤2%). Itis evident fromtheresults of accuracy study, reported in [Table 4] that the proposed method enablesvery accurate quantitative simultaneous estimation of CEF and MOX

Robustness and Ruggedness

Robustness studies were carried out after deliberate alterations of flow rate and mobile phase compositions and pH. It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak interest. The degree of reproducibility of the results has proven that the method is robust and the data are summarized in [Table 5]. The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Shimadzu HPLC and by two different operators using different columns of similar type like Phenomenex C_{18},HypersilC_{18} and the results were shown in [Table 6],the low RSD values confirms the ruggedness of the method.

Estimation of cefixime and moxepril in tablet dosage form

Twenty tablets of each brand Suprax® and Moxif®were weighed individually and ground to a fine powder. An accurately weighed powder sample equivalent to 100 mg of both CEF and MOX were transferred to 100 ml of volumetric flask

Table 6: Robustness testing of the method

Parameter	Modification	Cefixime % Recovery	Moxifloxacin % Recovery
pH	6.2	99.63	99.54
	6.0	99.57	99.82
	5.8	99.92	101.06
Buffer Composistion(A)	38	99.84	100.23
	40	101.6	100.21
	42	100.4	99.79
Flowrate (mL/min)	0.9	101.0	99.61
	1.0	99.31	99.84
	1.1	101.0	99.32

Table 7: Analysis of formulation

Drug	Labeled amount(mg)	Amount of mg/tab found*	%Label claim	%RSD
Cefixime	40	39.8	99.53	0.312
Moxifloxacin	40	40.21	100.02	0.115

*Averageof sixdeterminations

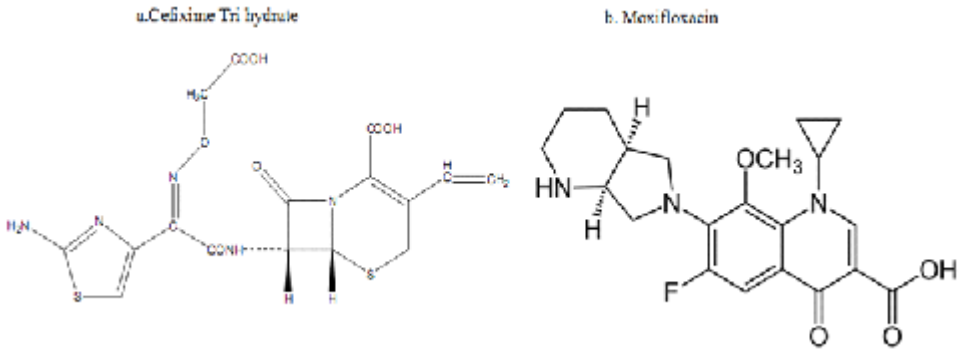

Fig. 1: Structure of Cefixime trihydrate and Moxifloxacin

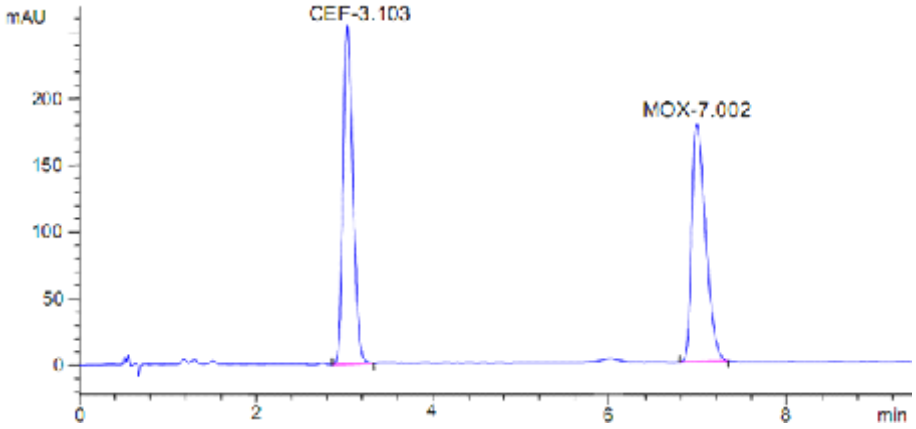

Fig. 2: Typical chromatogram of Cefixime and Moxifloxacin

anddissolvedin25mL of a 45:60 v/v mixture of acetonitrile and phosphate buffer. The contents of the flask were sonicated for 15 min and a further 25mL of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a0. 45µ membrane filter.This solution was further diluted toget the required concentrations. The solution containing 40µg/ml was injected in to the column sixtimes. The average peak area of the drugs was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in[Table7].

CONCLUSION

The developed method is accurate, simple, economical, rapid and selective for the simultaneous estimation of cefixime and moxifloxacin in bulk and in tablet dosage form without prior separation. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The sample preparation is simple,the analysis time is short and the elution is isocratic.Hence, the proposed method can be conveniently adopted for the routine quality control analysis in the combination formulations.

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