International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

DEVELOPMENT AND VALIDATION OF NOVEL HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME AND MOXIFLOXACIN IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, accurate and precise RP-HPLC method has been developed and validated for simultaneous estimation of Cefixime and Moxifloxacin in combined tablet dosage form. The chromatographic separation was carried out on Hypersil BDS C18 column (100 x 4.6 mm; 3 µ) with a mixture of phosphate buffer pH 6.0: acetonitrile (75: 25 V/V) as a mobile phase; at a flow rate of 1.0 mL/min. UV detection was performed at 293 nm. The retention times were 2.374 min and 5.776 min for Cefixime and Moxifloxacin respectively. Calibration plots were linear (r^2=0.999) over the concentration range of 5-30 µg/mL for both Cefixime and Moxifloxacin. The method was validated for linearity, accuracy, precision, specificity and sensitivity. The proposed method was successfully used for quantitative analysis of Cefixime and Moxifloxacin tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that the method is specific, rapid, reliable and reproducible. The high recovery and low relative standard deviation confirm the suitability of the proposed method for routine estimation of Cefixime and Moxifloxacin in pure sample and tablet dosage forms.

Keywords: Cefixime, Moxifloxacin, HPLC, Validation.

INTRODUCTION

Cefixime (Fig. 1) is a semisynthetic cephalosporin antibiotic used to treat infections caused by bacteria[1]. Cefixime treat infections of the ear, sinuses, throat, chest and lungs. Chemically, it is (6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2ene-2-carboxylic acid, 7[2]-(Z)-[O-(carboxy methyl) oxime] trihydrate[2]. The bactericidal action of cefixime due to the inhibition of cell wall synthesis. It binds to one of the penicillin binding proteins (PBPs) which inhibits 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.

Moxifloxacin hydrochloride (Fig. 2) is a fourth generation synthetic antibacterial agent[3]. Chemically, it is l-Cyclopropyl-6-fluoro-l,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolol [3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid, mono hydrochloride[4]. Moxifloxacin is used to treat infections including, respiratory tract infections, cellulitis, anthrax, intraabdominal infections, endocarditis, meningitis and tuberculosis. Moxifloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication.

Literature survey reveals that few spectrophotometric[5-8] and HPLC[9-10] methods were reported for simultaneous estimation of Cefixime and Moxifloxacin in combination dosage form. Therefore, an attempt has been made to develop an accurate, rapid and reproducible RP-HPLC[11] method for simultaneous determination of Cefixime and Moxifloxacin in tablet dosage form and validate it, in accordance with ICH[12] guidelines.

MATERIALS AND METHODS

Chromatographic conditions: Separation was performed with Waters HPLC equipped with a pump 2695, auto sampler and UV detector. Empower2 software was applied for data collecting and processing. The separation was achieved on a Hypersil BDS C18 column (100 x 4.6 mm, 3 µ). The mobile phase consisted of phosphate buffer pH 6.0: acetonitrile in the ratio of 75: 25 V/V. The flow rate was 1.0 mL/min and UV detection was performed at 293 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µ membrane filter. The injection volume was 20 µL and all the experiments were performed at temperature 30˚C. The run time was set at 12 min.

Chemicals and reagents: Pharmaceutical grade of Cefixime and Moxifloxacin were kindly supplied as gift samples by Chandra Labs, Hyderabad, India. Commercially formulations of tablets were purchased from local market. Tablets claimed to contain 400 mg of Cefixime and 400 mg of Moxifloxacin have been utilized in the present work. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. HPLC grade acetonitrile was purchased from E. Merck (India) Ltd., Mumbai, India. HPLC grade water obtained from Milli Q water purification system was used throughout the study.

Preparation of standard solution: Accurately weigh and transfer 20 mg of Cefixime and 20 mg of Moxifloxacin working standard into 100 mL volumetric flask, add about 60 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent (stock solution), from this stock solution pipette out 10 mL into 100 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µ filter.

Preparation of sample preparation: Twenty tablets were accurately weighed, their mean weight was determined and they were mixed and finally powdered. Transfer the sample equivalent to 20 mg of Cefixime and 20 mg of Moxifloxacin into 100 mL volumetric flask. Add about 60 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µ filter, from this stock solution pipette out 10 mL into 100 mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45 µ filter.

METHOD VALIDATION

The method was validated in accordance with ICH guidelines. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility and robustness etc.

Linearity: Several aliquots of standard solutions of Cefixime and Moxifloxacin were taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase such that the final concentrations were 5-30 µg/mL for both Cefixime and Moxifloxacin. Evaluation of the two drugs was performed with UV detector at 293 nm, peak area was recorded for all the peaks. The correlation coefficient values were R^2=0.999 for both Cefixime and Moxifloxacin. The results showed that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated.

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed HPLC method. The LOD for Cefixime and Moxifloxacin were found to be 0.964 µg/mL and 0.815 µg/mL respectively. The LOQ for Cefixime and Moxifloxacin were found to be 2.922 µg/mL and 2.471 µg/mL respectively.

Accuracy: The accuracy of the method was assessed by recovery studies of Cefixime and Moxifloxacin in the combined dosage form at three concentration levels. A fixed amount of preanalyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The contents of Cefixime and Moxifloxacin per tablet were calculated. The % mean recoveries of Cefixime and Moxifloxacin were 100.13% and 99.68% that shows there is no interference from excipients and the lower values of RSD of assay indicate the method is accurate.

Precision: The precision was determined for both the drugs Cefixime and Moxifloxacin in terms of intra-day and inter-day precision. For intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and %RSD for Cefixime and Moxifloxacin were 0.56% and 0.13% respectively (limit %RSD < 2.0%). In addition, the inter-day precision was studied by injecting the same concentration of standard solution on consecutive

days and the %RSD for Cefixime and Moxifloxacin were 0.14% and 0.12% respectively (limit %RSD < 2.0%).

Ruggedness: The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types, which demonstrated that the developed HPLC method is rugged.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions like mobile phase composition and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method so developed is robust.

Specificity: Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances. No interference from any of the excipients was found at retention times of the examined drugs. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed method.

Assay: 20 µL of each standard and sample solution were injected and from the peak area of Cefixime and Moxifloxacin, amount of each drug in samples were computed. The result of assay undertaken yielded 99.74% and 99.79% of label claim of Cefixime and Moxifloxacin respectively.

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop an accurate assay method for simultaneous estimation of Cefixime and Moxifloxacin in tablet dosage form using Hypersil BDS C18 column (100 x 4.6 mm, 3 µ) in isocratic mode with mobile phase composition of phosphate buffer pH 6.0: acetonitrile in the ratio of 75: 25 V/V. The use of phosphate buffer pH 6.0: acetonitrile in the ratio of 75: 25 V/V resulted in peak with good shape and resolution. The flow rate was 1.0 mL/min and both the components were measured with UV detector at 293 nm. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 5 to 30 µg/mL for both Cefixime and Moxifloxacin with correlation coefficient of 0.999 for both Cefixime and Moxifloxacin. Linear regression data for Cefixime and Moxifloxacin were given in Table 2 and the linearity curves for Cefixime and Moxifloxacin were shown in Fig. 3 and Fig. 4.

The % mean recoveries were found to be 100.13% for Cefixime and 99.68% for Moxifloxacin, which indicate the method is accurate. The accuracy results were shown in Table 3. The %RSD for intra-day precision and inter-day precision for Cefixime were found to be 0.56 and 0.14 and for Moxifloxacin were found to be 0.13 and 0.12 respectively, which indicate the method is precise. The precision results were shown in Table 4 and Table 5.

The retention time of Cefixime and Moxifloxacin was 2.374 min and 5.776 min respectively. The number of theoretical plates calculated was 4898 for Cefixime and 5534 for Moxifloxacin and symmetry factor was 1.28 for Cefixime and 1.27 for Moxifloxacin, which indicates efficient performance of the column. The LOD for Cefixime and Moxifloxacin were found to be 0.964 µg/mL and 0.815 µg/mL respectively. The LOQ for Cefixime and Moxifloxacin were found to be 2.922 µg/mL and 2.471 µg/mL respectively, which indicate the sensitivity of the method. The summary of system suitability parameters were shown in Table 6. Validated method was applied for the determination of Cefixime and Moxifloxacin in commercial formulations. The % assay was found to be 99.74% and 99.79% for Cefixime and Moxifloxacin respectively and the assay results were shown in Table 7.

Typical chromatogram of standard showing the separation of the drugs Cefixime and Moxifloxacin was shown in Fig. 5. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

The developed HPLC method is simple, specific, accurate and precise for the simultaneous estimation of Cefixime and Moxifloxacin in tablet dosage form. The developed method provides good resolution between Cefixime and Moxifloxacin. It was successfully validated in terms of linearity, accuracy, precision, specificity, robustness, LOD, LOQ and system suitability in accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs in combinations.

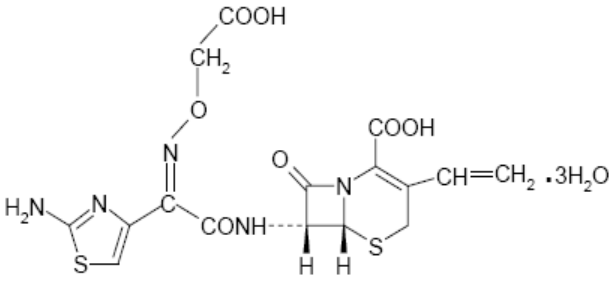

Fig. 1: Chemical structure of Cefixime

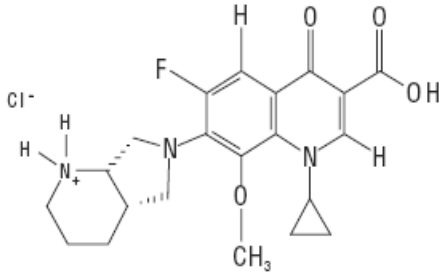

Fig. 2: Chemical structure of Moxifloxacin Hcl

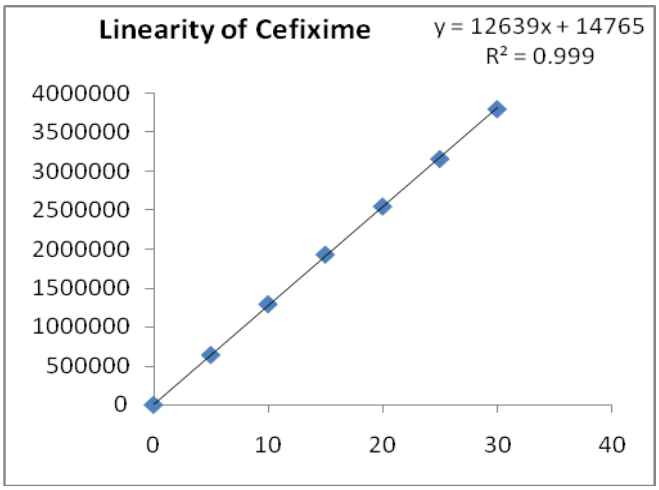

Fig. 3: Linearity curve of Cefixime

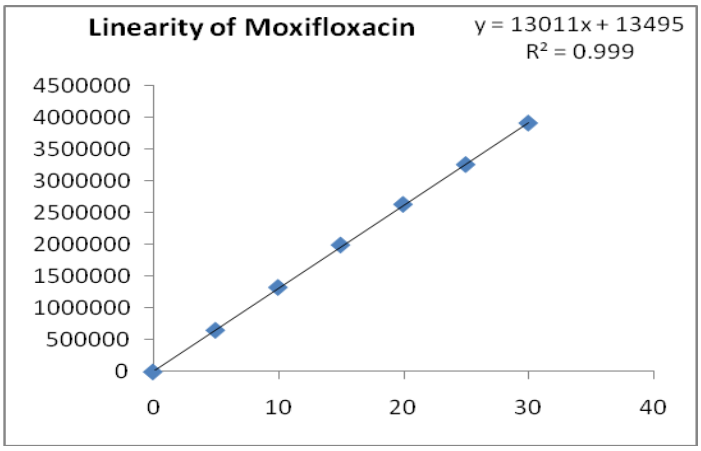

Fig. 4: Linearity curve of Moxifloxacin

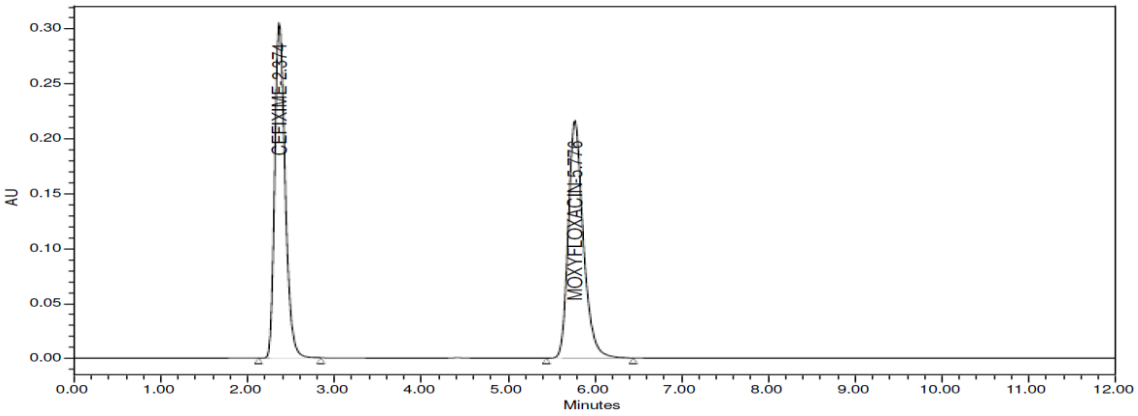

Fig. 5: Typical chromatogram of standard for Cefixime and Moxifloxacin

Table-1: Optimized chromatographic conditions of Cefixime and Moxifloxacin

Parameter	Condition
Mobile phase	Phosphate buffer: acetonitrile (75: 25, V/V)
pH	6.0
Diluent	Mobile phase
Column	Hypersil BDS C18 (100 x4.6 mm, 3 µ)
Column temperature	30^0C
Wave length	293 nm
Injection volume	20 µL
Flow rate	1.0 mL/min
Run time	12 min

Table-2: Linearity results of Cefixime and Moxifloxacin

Concentration of	Area	Concentration of Moxifloxaci	Area
5	640777	5	653362
10	1293361	10	1325728
15	1930698	15	1991277
20	2549850	20	2628297
25	3160196	25	3254088
30	3800023	30	3904215

Table-3: Accuracy data for proposed method

Spiked evel	Amount added (mg)		Amount found (mg)		% Recovery	
	Cefixime	Moxifloxacin	Cefixime	Moxifloxacin	Cefixime	Moxifloxacin
50%	50	50	50.62	50.02	101.24	100.05
100%	100	100	99.69	99.99	99.69	99.99
150%	150	150	149.21	148.53	99.47	99.02

Table-4: Intra-day precision data of proposed method

S. No.	Cefixime	Moxifloxacin
1	2542125	2601216
2	2544376	2594536
3	2545587	2596534
4	2576435	2592156
5	2567564	2598657
6	2548768	2594356
Average	2554130	2596243
SD	14244	3280
%RSD	0.56	0.13

Table-5: Inter-day precision data of proposed method

S. No.	Cefixime	Moxifloxacin
1	2543407	2600495
2	2546721	2596915
3	2546296	2594821
4	2551278	2595050
5	2552590	2593261
6	2551653	2591098
Average	2548657	2595273
SD	3693	3215
%RSD	0.14	0.12

Table-6: System suitability parameters of proposed method

Parameters	Cefixime	Moxifloxacin
Linearity (µg/mL)	5-30	5-30
Theoretical plates	4898	5534
Symmetry factor	1.28	1.27
Resolution	0.00	13.16
Retention time (min)	2.374	5.776
LOD (µg/mL)	0.964	0.815
LOQ (µg/mL)	2.922	2.471

Table-7: Assay results of proposed method

Formulation	Label claim		Amount found		%Assay	
Formulation 1	**Cefixime**	**Moxifloxacin**	**Cefixime**	**Moxifloxacin**	**Cefixime**	**Moxifloxacin**
	200 mg	200 mg	199.4 mg	199.5 mg	99.74%	99.79%

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