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Method development and validation for the quantitative estimation of cefixime and ofloxacin in Pharmaceutical preparation by RP- HPLC

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ABSTRACT

A simple, rapid, selective, precise and economical RP-HPLC method has been developed and validated for the quantitative estimation of Cefixime and Ofloxacin in pharmaceutical preparation. Chromatographic separation was achieved on using Kromasil C_{18} column (250 mm x 4.6 mm, 5µm i.d) analytical column with mobile phase consisting of 40:60 v/v mixture of Ammonium Acetate buffer: Acetonitrile. flow rate was 1.0 ml/min and the detection of wavelength was 294nm. In the developed method Cefixime and Ofloxacin elute at typical retention times of 2.26 min and 3.24 min respectively. The proposed method has permitted the quantification of Cefixime in the linearity range of 60 -140 µg/mL and for Ofloxacin in the range of 60 - 140 µg/ mL. The intraday and interday precision was found less than 2% and the LOD and LOQ for Cefixime were found to be 0.146 - 0.44 µg/mL and for Ofloxacin were found to be 0.16 - 0.49 µg/mL respectively. The validated optimized method for analysis of Cefixime and Ofloxacin as per ICH Q2B guidelines was found to be simple, precise and reproducible. Undoubtedly present developed easiest, rapid and validated method can be applied routinely for the analysis of drugs in bulk as well as tablet dosage form.

Key words: Cefixime, Ofloxacin, RP-HPLC, Simultaneous Quantification.

INTRODUCTION

Cefixime (6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl) glyoxylamido] -8-oxo-3-vinyl-5- thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-(Z)-[o-(carboxymethyl)-oxime] (Fig. 1) is an Antibacterial drug (β-lactamase inhibitor-cephalosporin antibiotic) Is an orally absorbed drug [1-3]. It acts by interfering in the synthesis of bacterial cell wall. It is not hydrolised in the common plasmid or by chromosomal β-lactmase enzyme. It binds to specific penicillin binding proteins (PBPS) located inside the bacterial cell wall causing the inhibition of the third and last stage of bacterial cell wall synthesis.

Ofloxacin (fig 2) is an antiinfective drug It acts by targeting bacterial DNA gyrase and topoisomerase IV. It prevents the supercoiling of DNA during replication or transcription [4-6]. By inhibiting their function ofloxacin thereby inhibits normal cell division. Ofloxacin is used to treat pneumonia and bronchitis caused by influenza, skin infections, gonorrhea and chlamydia, urinary tract and prostate infections.

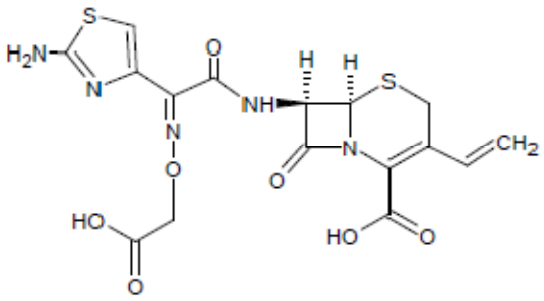

Fig. 1: Chemical structure of Cefixime

So this combination is used for the treatment of typhoid fever, urinary tract infection, respiratory tract infection, nosocomial infections, soft tissue infections, surgical prophylaxis and intra-abdominal infections [7].

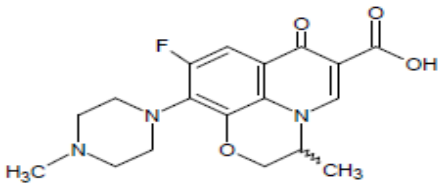

Fig 2: Chemical structure of Ofloxacin

Cefixime and Ofloxacin in spectrophotometric and few HPLC techniques are reported for the determination of Cefixime and Ofloxacin in pharmaceutical dosage form, and most of them used different buffers as a mobile phase which is reducing the life span of an analytical column and preparation of buffer with the maintenance of proper pH is cumbersome process [8-15] . The above fact indicates there is need to develop a sensitive, stable and accurate method, the novelty of the present method involves the use a chief, simple solvent and well separated drug under study in presence of different degrading products. So the present RP-HPLC method for the simultaneous determination of Cefixime and Ofloxacin in bulk and tablet dosage form can be used in the quality control laboratory for routine analysis.

MATERIALS AND METHODS

Chromatographic conditions

The mobile phase was prepared by mixing Acetate buffer and Acetonitrile filtered through a 0.45 µm membrane filter and degassed by using an ultrasonicator for 15 min prior to use. The chromatographic separation was achieved on using Kromasil C_{18} column (250 mm x 4.6 mm, 5µm i.d) analytical column. The system equilibrated for 30 min and analysis was carried out under isocratic conditions using a flow rate of 1.0 mL min-1. Chromatograms were recorded at 294 nm and the injection volume was 10 µL.

Materials

Cefixime (CEF) and Ofloxacin (OFL) standard drugs were obtained from associated biotech labs. HPLC-grade Acetonitrile were obtained from Sigma-Aldrich company and Acetate buffer of Analytical reagent grade was obtained from Merck, HPLC grade water was also obtained from Merck.

Instrumentation

The liquid chromatographic system (LC-100) was carried out using Kromasil C_{18} column (250 mm x 4.6 mm, 5µm i.d) analytical column. A mobile phase of (40:60) v/v mixture of Ammonium Acetate buffer: Acetonitrile as pumped at a low rate of 1 mL. min-1.

Preparation and selection of mobile phase:
The preliminary isocratic studies on a reverse phase C_{18} column with different mobile phase combination of acetonitrile and phosphate buffer pH 7.0±0.1 were studied for separation of both drugs. The optimal composition of mobile phase determined to be acetonitrile: ammonium acetate buffer (60:40) v/v and the pH was adjusted to 5 by addition of 10% Ortho phosphoric acid and was filtered through 0.45 µm membrane filter.

Preparation of combined working standard stock solution
Stock solution of standard drugs was prepared by weighing accurately 10 mg of CEF, 10 mg of OFL taken in a 50 mL standard flask. To it 50 mL of the mobile phase was added and sonicated for 15 minutes to dissolve the drugs. The volume was made upto 100 mL with the mobile phase. It is sonicated for 20 min and filtered through 0.45 µm membrane filter. Both the solutions consist of 100 µg/mL concentration each.

Assay of Marketed formulation
Preparation of working test stock solution
Twenty tablets were weighed and powdered. Tablet powder having weight equivalent to 10 mg of CEF (CEF 200 mg, OFL 200 mg label claim HIFEN PLUS (HETERO HEALTH CARE)) was weighed accurately and taken in a 100 mL volumetric flask. To it 50 mL of the mobile phase was added and sonicated for 20 min minutes to dissolve the drugs. The volume was made upto 100 mL with mobile phase. The resulting solution was then filtered through a 0.45 µm membrane filter to prepare a stock solution of the tablet sample. Both the drugs consist of 1000 µg/mL concentration.

Preparation of calibration curves
Solutions of both drugs having different concentrations in a linear range were prepared by dilution of the standard solutions by the mobile phase. These solutions (20 µL) were injected into the HPLC system, chromatographed and peak areas were measured, peak areas were then plotted against the respective concentrations for both CEF and OFL.

Analysis of Tablet dosage form
Six replicates of the required dilutions were prepared from capsule stock solution and sonicated for 10 min. These solutions (20 µL) were injected for quantitative analysis. The amounts of CEF and OFL per tablet were calculated by extrapolating the peak area from the calibration plot. Results of analysis are reported in Table 1. Precision was measured both intra-day and inter-day. In the intra-day study the concentration of both drugs were calculated three times on the same day at intervals of an hour. In the inter-day study the concentrations of both drugs were measured on three different days.

Table 1: Analysis of marketed Formulation

Formulation	Label claim (mg)	Amount found(mg)	% Assay
Hyfen plus	CEF 200 mg	198.28*	99.14
	OFL 200 mg	199.02*	99.51

** Average of three readings.*

Recovery studies: To perform the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. The results of the analysis are shown in Table 2 and 3.

Table 2: Recovery study of CEF

Concentration of Spiked level		Amount present (mg)	Amount added (mg)	Amount found (mg)	%Recovery	Mean Recovery
80	80	20	101.1	101.1		
100	100	20	119.9	99.9	100.5	
120	120	20	141.1	100.7		

Table 3: Recovery study of OFL

Concentration of Spiked level		Amount present (mg)	Amount added (mg)	Amount found (mg)	%Recovery	Mean Recovery
80	80	20	99.8	99.8		
100	100	20	120.6	100.5	100.1	
120	120	20	140.2			

Validation parameters

Validation of the optimized HPLC method was done with respect to following parameters as per ICH norms [16].

System suitability

This study was carried out to verify that the analytical system is working properly and can give accurate and precise results. It was carried out by injecting standard solutions of 100 µg/mL of CEF and OFL six times. The system suitability parameters like theoretical plates, peak area, retention time and asymmetric factor were evaluated (Table 4).

Table 4: Results for system suitability parameters of CEF

Parameters	CEF	OFL
λmax (nm)	291 nm	297 nm
Beer's law limit	60-140µg/mL	60-140µg/mL
Correlation coefficient	0.998	0.999
Retention time (min)	2.26 min	3.24
Theoretical plates	2692.5	4890.67
Tailing factor	1.88	1.58
Limit of Detection (µg/ml)	0.146 µg/mL	0.16 µg/mL
Limit of Quantitation (µg/ml)	0.44 µg/mL	0.48 µg/mL

Precision

The intra-day precision was determined by analyzing the CEF and OFL for six times on same day (intra-day study). The chromatograms were recorded (Table 5).

Table 5 intra day precision of CEF and OFL

Injection	RT CEF	RT OFL	Peak area CEF	Peak area OFL	TP CEF	TP OFL
1	2.307	3.307	3293	5356	2948	5006
2	2.30	3.293	3283	5319	2931	4966
3	2.287	3.273	3262	5294	3100	4906
4	2.27	3.253	3252	5274	2855	4846
5	2.283	3.267	3235	5239	3091	4886
6	2.28	3.257	3235	5238	2880	4856
Mean	2.28	3.275	3260	5286	2967	4911
SD	0.013	0.02	24.39	46.27	54	63.16
%RSD	0.5	0.6	0.7	0.8	1.8	1.2

RT: retention time, TP: tailing factor

Specificity

The specificity study was done to check the interference of extraneous components for that a solution containing a mixture of tablet and standard was prepared using sample preparation procedure and injected into the system, to evaluate possible interfering peaks.

Linearity and range

This study was Performed by preparing the concentrations in the range of 60-140µg/mL for CEF and 60-140 µg/mL for OFL by diluteing the standard stock solution at different levels (Table 6). Peak area and retention time was studied and constructed the calibration curve by plotting concentrations and peak area to check the correlation.

Table 6: Linearity of CEF and OFL

S.No	Concentration	Peak area of CEF	Peak area of OFL
1	60	2041	3225
2	80	2551	4227
3	100	3180	5154
4	120	3651	6054
5	140	4234	7006

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated.

Robustness

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 ml/min to 0.8 ml and 1.2ml/min. The wavelength was changed from 294nm to 296nm and 292nm.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

From the slope and standard deviation LOD and LOQ was calculated. Six replicates of the analyte were prepared in the range of 60-140 µg/mL for CEF and 60-140 µg/mL for OFL. The limit of detection was defined as the concentration for which a signal-to-noise ratio of 3 was obtained and for quantitation limit; a signal-to-noise ratio of 10 was considered.

RESULTS

The HPLC method was found to be simple, accurate, economic and rapid for routine simultaneous estimation of CEF and OFL in combined tablet dosage form at 294 nm. The regression: 0.998 and 0.999, intercept: 380.4 and 2316 and slope: 27.43 and 938.9 were found to be for CEF and OFL respectively. Recovery was in the range of 100.1–100.5% and shows the high precision of the developed method.

DISCUSSION

Acetate buffer: Acetonitrile (40:60) pH 5.0 showed less retention time with good peak symmetry was optimized as the mobile phase and optimized chromatogram showed in the figure 5.The optimized injection volume was 10 µL and detection wavelength 294 nm was selected. The analysis of CEF and OFL was achieved Kromasil C18 (250 mm x 4.6 mm , 3.5µm i.d) using Acetate buffer: Acetonitrile (40:60) pH 5.0 as mobile phase with the retention time of CEF and OFL as 2.26 min and 3.24 min respectively. The developed method was found to be specific and validated as per ICH guidelines.

Amongst the various mobile phases used, acetonitrile: water: tri ethylamine (30:67:3) v/v and the pH was adjusted to 5 by addition of 10% Ortho phosphoric acid was found to be robust at 2ml/min flow rate. Mobile phase and flow rate selection was based on peak parameters such as height, tailing, theoretical plates, capacity factor, run time, resolutions. A typical chromatogram of CEF and OFL is shown in Fig.3, 4. The optimum wavelength for detection was 294 nm at which detector response was obtained best. The average retention time for CEF and OFL was found to be 2.26 min. and 3.24 min respectively. They are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out and its results are shown in Table 4.Therefore from the above experimental data it can be well concluded that the developed simultaneous method is stable, accurate and economic and validated has ever developed and indicates the suitability of the method for the routine analysis of CEF and OFL bulk and tablet dosage form.

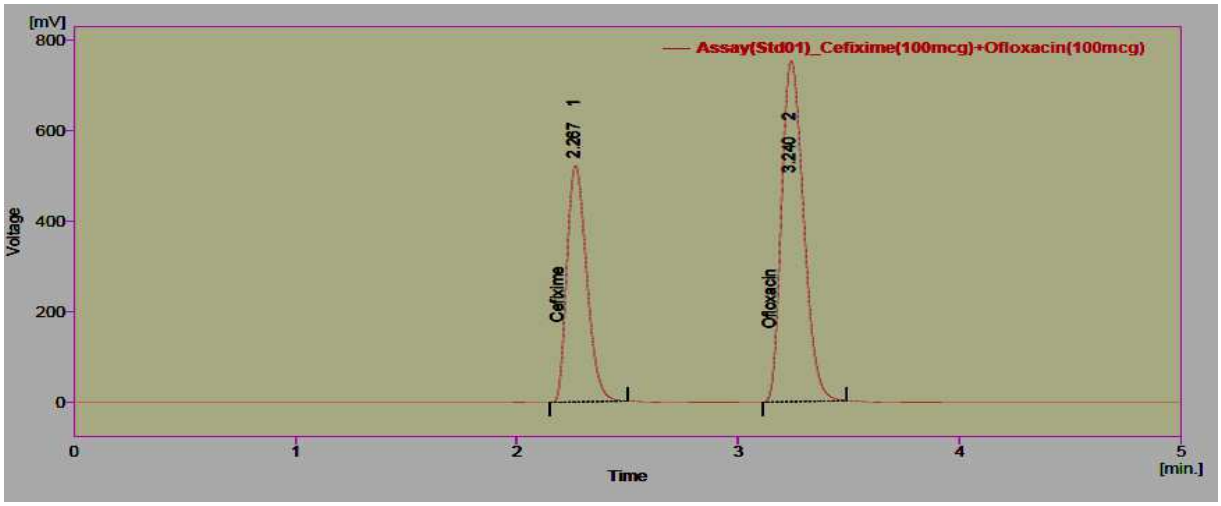

Fig 3: Chromatogram of CEFand OFL standard solution

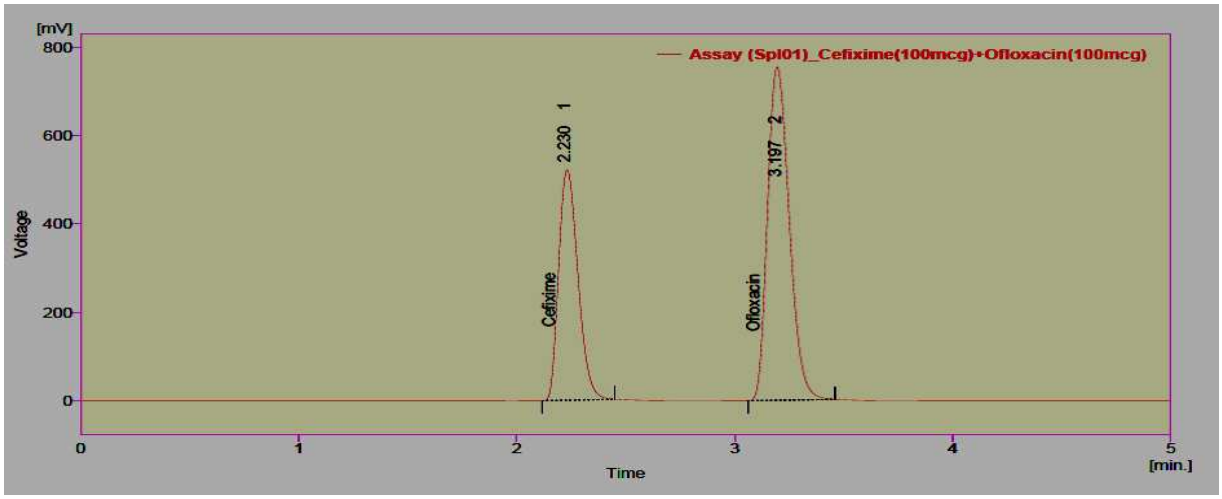

Fig 4: Chromatogram of CEF and OFL test solution

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