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STABILITY INDICATING RP- HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CEFIXIME AND OFLOXACIN FROM PHARMACEUTICAL DOSAGE FORMULATION

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ABSTRACT

In present study, a successful attempt has been made to develop a simple, sensitive and validated RP-HPLC method for the simultaneous determination of Cefixime and Ofloxacin from combined pharmaceutical drug formulation. Chromatographic separation of Cefixime and Ofloxacin was achieved with gradient elution on C-18 column with mobile phase A- 0.1% formic acid in Water and mobile phase B- 0.1% formic acid in Methanol at a wavelength 250 nm. The method was validated in the terms of its linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. Linearity of the method was found to be in the concentration range of 5-200 µg/mL for Cefixime and 5-200 µg/mL for Ofloxacin with correlation coefficient greater than 0.999 for both the analytes. The total eluting time for the both components is less than seven minutes. Proposed method was found to be simple, precise, and accurate and can be successfully applied for routine quality control analysis and simultaneous determination of Cefixime and Ofloxacin in combined pharmaceutical drug formulations.

KEYORDS: RP-HPLC, Cefixime, Ofloxacin, Pharmaceutical drug formulations and Validation.

INTRODUCTION

In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, are the most common. Drugs with

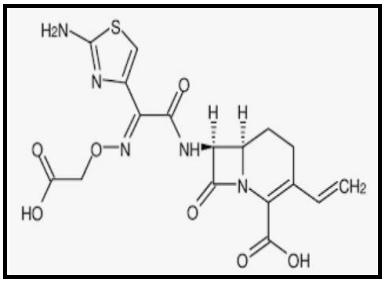
antifungal and antiprotozoal activity have been used in the treatment of the same. In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Many of these antibacterial drugs are found in combination with antifungal and antiprotozoal drugs which are highly effective against fungal and protozoal infections. **Cefixime,** $C_{16}H_{15}N_5O_7S_2$ that is (6*R*,7*R*)-7-[[(2*Z*)-2-(2-amino-1,3-thiazol-4-yl)-2 (carboxymethoxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2- carboxylic acid. (Molecular weight:- 453.5 g/mol)] is used in the treatment of bacterial infection. Cefixime is a broad-spectrum, third-generation cephalosporin antibiotic derived semi synthetically from the marine fungus Cephalosporium acremonium with antibacterial activity. **Ofloxacin,** $C_{18}H_{20}FN_3O_4$ that is 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.$0^{5,13}$]trideca-5(13),6,8,11-tetraene-11-carboxylicacid; hydrochloride is second generation fluroquinolone with an antibacterial spectrum of activity used as an antibacterial drug. (Molecular weight: - 361.4 g/mol) **Ofloxacin** is a quinolone antibiotic useful for the treatment of a number of bacterial infections.

A literature survey has revealed very few chromatographic and spectrophotometric methods. In the present work we have focused on deciding the optimum chromatographic conditions for the simultaneous determination of Cefixime and Ofloxacin in combined pharmaceutical drug formulations and successfully developed completely novel validated method for the same.

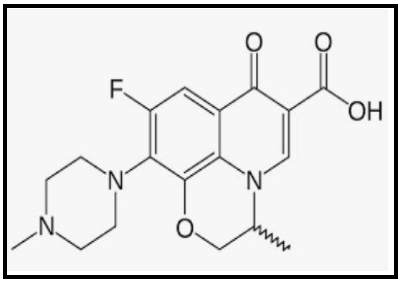
OBJECTIVE

The main objective of study is to provide a simple, rapid, efficient, reliable and economic method for the simultaneous determination of Cefixime and Ofloxacin in combined pharmaceutical formulations using RP-HPLC. The proposed developed method to be subsequently validated as per ICH guidelines.[8-9]

STRUCTURE



Cefixime



Ofloxacin

MATERIAL AND METHODS

Chemicals and Reagents

Standard Cefixime and Ofloxacin were obtained from local pharmaceutical company with claimed purity above 99.0%. All the solutions were prepared in double distilled water. All the necessary reagents used i.e. water and methanol (HPLC grade). Mobile phase was filtered using 0.45µm syringe filter made by Millipore whereas; Whatman's filter paper No.41 (purchased from local market) was used in the preparation of sample solution.

Apparatus and Chromatographic Conditions

The Shimadzu LC2010 is a high-performance liquid chromatographic system with a quaternary, low-pressure mixing pump inline vacuum degassing and PDA Detector (at wavelength 250 nm) with Chromeleon software.

Chromatographic Mode	Gradient
Column	Agilent Poroshell EC C-18, 50 mm length x 4.6 mm ID 2.7µm particle size
Wavelength	250nm
Column oven temperature	40°C
Autosampler temperature	5°C
Injection Volume	5.0 µl
Flow rate	2.0 ml/min
Mobile Phase:	Mobile phase A- 0.1% formic acid in Water Mobile phase B- 0.1% formic acid in Methanol Filter and Degas
Diluent	Water: Methanol (950:50)

Praparation of Standard Solution

Weigh accurately 20 mg of Cefixime WS and 20 mg Ofloxacin WS transfer it into a 50 ml standard flask, add 35 ml of diluent and sonicate to dissolve. Allow it to cool at room temperature, mix well and make up to the volume with diluent. The working standard solution 100 µg/mL of Cefixime and 100 µg/mL of Ofloxacin were prepared by diluting 5 ml of this solution in to a 20 ml standard flask, mix and dilute up to the volume with diluent.

Praparation of Sample Solution

Commercial brand containing of Cefixime and Ofloxacin in combination was procured. Each brand contained a label claim of 200 mg of Cefixime and 200 mg of Ofloxacin per tablet. Five tablets of each brand were weighed and powdered for the analysis. The powder (748mg) equivalent to 200 mg of Cefixime and 200 mg of Ofloxacin was accurately weighed, transferred into 200ml standard flask; add 170 ml of diluent and sonicate to dissolve. Allow it

to cool at room temperature, mix well and the mixture was sonicated for 30 mins, finally volume of the solution was made up to 200 mL with diluent. The solution was filtered through Whatman filter paper no 41. and appropriate volume (5.0 mL) of stock solution was diluted to 50 mL with the diluent to obtain a solution containing 100 µg/mL of Cefixime and 100 µg/mL of Ofloxacin.

ANALYTICAL METHOD VALIDATION[8-9]

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. System suitability has been checked by recording Theoretical plates and Tailing factor for both OFX and CFX which is given in **Table.1**

Specificity

The specificity of method was confirmed by observing the chromatograms of both the combined standard solution and the drug sample solutions. The chromatograms obtained from the drugs sample solution were found to be identical to those obtained for standard solution. The addition of the standard solution to the drug sample solution did not change the characteristics of chromatograms. This gives the validity of method for the determination of both the drugs from combined pharmaceutical formulation.

Linearity and Range

The linearity for Cefixime and Ofloxacin was observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 5 µg/mL to 200 µg/mL for Cefixime and 5 µg/mL to 200 µg/mL for Ofloxacin. The calibration curves were constructed with concentration (C) against peak area. The slope, intercept, regression equation and correlation coefficient for the OFX and CFX was obtained is given in **Table.1 and Figure.2**

LOD AND LOQ

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. LOD and LOQ for Cefixime were 3.0 µg/mL and 10.0 µg/mL and for Ofloxacin were found to be 3.0 µg/mL and 10.0 µg/mL respectively is given in **Table.1**

Intraday and Interday Precision

The intra-day and inter-day precision was used to study the variability of the method. It was checked by recording the chromatograms of sample solutions of Cefixime and Ofloxacin at three different levels i.e. 50% ,100% and 150% both at intra-day (five times within 24 hour) and inter-day (two times each. during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision was found to be less than 1.0% for both OFX and CFX. Result of intra and inter day precision studies are given in **Table.1**

Assay

The developed chromatographic method was used for simultaneous determination of Cefixime and Ofloxacin from commercial brand of formulation. The sample solutions were analyzed by the developed method described above. Chromatograms were recorded under the optimum experimental conditions. Resulting peak area of Cefixime and Ofloxacin were measured and the amount of Cefixime and Ofloxacin calculated using already constructed calibration graph. Result of assay studies are given in **Table.2**

Robustness

The robustness of the method was examined by the consistency of peak height and peak shape with the deliberately small changes in the experimental parameter. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition of mobile phase, change in flow rate and change in oven temperature. The chromatographic parameters of each analyte such as retention time, tailing factor, resolution and theoretical plates were measured at each changed condition. In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following two factors were selected for change: flow rate of the mobile phase (2.0 ± 0.2 mL/min) and column oven temperature (40 ± 2°C). One factor at the time was changed to estimate the effect. The solutions containing 100 µg/mL of both the drugs were applied onto the column. A number of replicate analyses (n = 3) were conducted for evaluation of each change of factors. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Accuracy (Recovery)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of varying weight of sample for sample preparation. A weight of sample was varied at different concentrations of preanalyzed sample solutions and analyzed by proposed method. The percentage recovery was determined at different levels i.e. from 50% to 150% level. The results of recovery analysis for Cefixime and Ofloxacin are shown in **(Table.3).**

RESULT AND DUSCUSSION

In the present work conditions were optimized for development and validation of a simple and accurate HPLC method for simultaneous quantification of Cefixime and Ofloxacin in combined pharmaceutical drug formulation. Method development was right from optimization of the condition and parameters i.e., selection of system, column, mobile phase, different composition of mobile phases have been tried. During optimizing the method, Methanol and Acetonitrile were choices as organic solvents. The cost of acetonitrile favored to choose methanol as solvent for further studies. The chromatographic conditions were optimized by using formic acid as a buffer for mobile phase preparation. After a series of screening experiments, it was concluded that gradient elution using formic acid and methanol gave better peak shapes and resolution, finally mobile phase A- 0.1% formic acid in Water and mobile phase B- 0.1% formic acid in Methanol is the most appropriate composition because both the components were eluted with good resolution and good peak shape. Under the described experimental conditions, sharp peaks that belong to OFX and CFX were obtained with gradient elution at retention time of 2.9 min and 3.2 min respectively. **(Figure.1)** The developed chromatographic method was validated using ICH guidelines. A new chromatographic method has been developed and subsequently validated for the simultaneous quantification of Cefixime and Ofloxacin from a combined drug formulation. The advantages of this method for analytical purposes lie in the rapid determination, its cost effectiveness, easy preparation of the sample, good reproducibility.

Table 1: Method validation parameters for the determination of Cefixime and Ofloxacin.

Parameters	Values	
	Ofloxacin	Cefixime
System suitability Theoretical Plates- Tailing Factor-	More than 22458 1.5	More than 35837 0.9
Linearity range (µg/mL)	5 to 200 µg/mL	5 to 200 µg/mL
Slope (m) [a)]	4170.24	5034.05
Intercept(c) [a)]	828.21	35.59
Correlation coefficient (R^2)	0.9999	0.9999
LOD (µg/mL)	3.0 µg/mL	3.0 µg/mL
LOQ (µg/mL)	10.0 µg/mL	10.0 µg/mL
Intraday precision (n=6)	0.1%	0.4%
Interday precision (n=6)	0.2%	0.5%
Assay	98.5% to 98.8%	100.1% to 101.0%
Recovery	98.5% to 99.6%	100.6% to 101.9%

Sample Details

Brand Name: ZIFI-O (FDC LIMITED)

Batch No.: 010I049

API: Ofloxacin-200 mg and Cefixime-200 mg

Excipients: q.s.

Colour: Tartrazine

Table 2: Result of Assay studies of Ofloxacin and Cefixime.

Brand name	ZIFI-O (FDC Limited)	
	Ofloxacin	Cefixime
Labeled claim (mg)	200mg	200mg
Drug found in mg	197.2 mg	200.9 mg
% RSD (n=3)	0.1	0.4
% Assay	98.6 %	100.5 %

Table 3: Results of Recovery studies of Ofloxacin and Cefixime.

Analyte	Level	Amount added for recovery study (in mg/mL)	Amount found in recovery study (in mg/mL)	RSD (%) (n = 3)	Recovery (%)	
					Minimum	Maximum
Ofloxacin	50%	50.0	49.80	0.3	99.0	99.6
	100%	100.0	99.30	0.1	99.3	99.6
	150%	150.0	148.35	0.3	98.5	99.1
Range					**98.5**	**99.6**
Cefixime	50%	50.0	50.60	0.3	101.2	101.9
	100%	100.0	100.6	0.2	100.6	101.0
	150%	150.0	151.8	0.2	100.7	101.2
Range					**100.6**	**101.9**

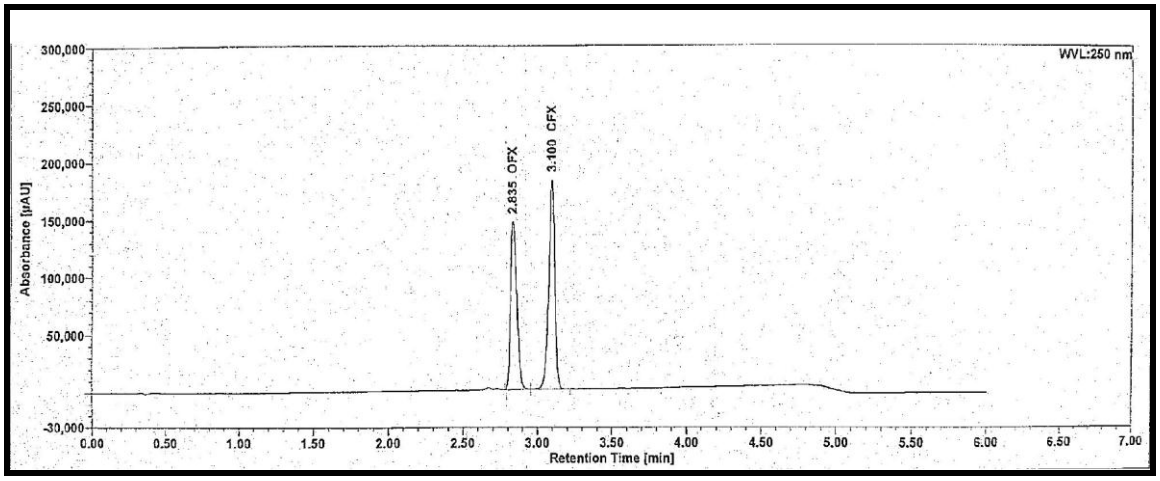
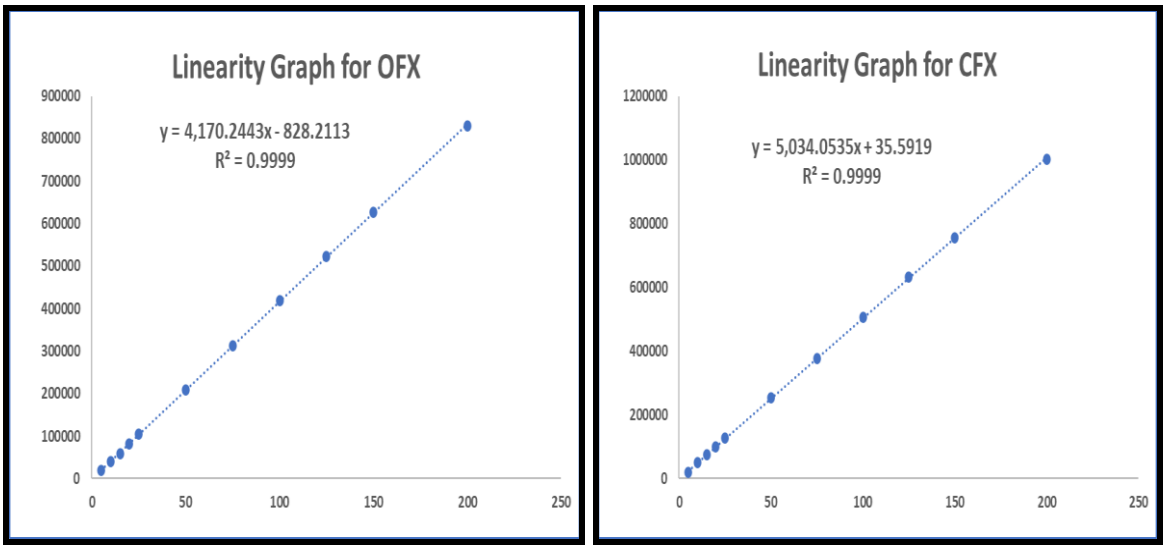


Figure 1: Chromatogram for Ofloxacin and Cefixime.



1. Ofloxacin Standard **2. Cefixime Standard**

Figure 2: Linearity graph for.

Y-axis – Peak Area

X-axis- Concentration of Drug in µg/mL

CONCLUSION

In addition to above mentioned points, the proposed method is found to be more simple, economic, accurate and practical. Thus, presented method can be recommended for simultaneous determination of Cefixime and Ofloxacin in routine quality control analysis in combined drug formulations.

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