

**STABILITY INDICATING RP- HPLC METHOD DEVELOPMENT AND VALIDATION  
FOR SIMULTANEOUS DETERMINATION OF CEFIXIME AND CLOXACILLIN FROM  
PHARMACEUTICAL FORMULATION****Pralhad Rege<sup>\*1</sup> and Avinash Jagdale<sup>2</sup>**<sup>1</sup>Assistant Professor, Dept. of Chemistry, St. Xavier's College, Mumbai.<sup>2</sup>Research Scholar, Dept. of Chemistry, St. Xavier's College, Mumbai.**Corresponding Author: Pralhad Rege**

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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 Cloxacillin from combined pharmaceutical drug formulation. Chromatographic separation of Cefixime and Cloxacillin 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 10-200 µg/mL for Cefixime and 25-500 µg/mL for Cloxacillin with correlation coefficient greater than 0.999 for both the analytes. The total eluting time for the both components is less than five 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 Cloxacillin in combined pharmaceutical drug formulations.

**KEYWORDS:** RP-HPLC, Cefixime, Cloxacillin, 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<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>7</sub>S<sub>2</sub> 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. **Cloxacillin**, C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S that is (2*S*,5*R*,6*R*)-6-[[[3-(2-chlorophenyl)-5-methyl-1,2-oxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate; is an antibiotic agent used for the treatment of beta-hemolytic streptococcal and pneumococcal infections as well as

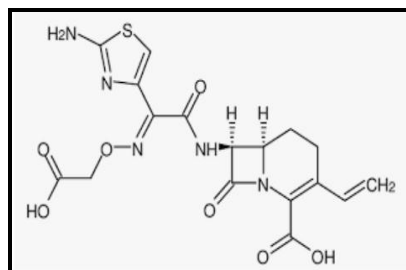
staphylococcal infections. (Molecular weight: - 435.9 g/mol) Cloxacillin is an antibiotic useful for the treatment of a number of bacterial infections. This includes impetigo, cellulitis, pneumonia, septic arthritis, and otitis externa.

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 Cloxacillin in combined pharmaceutical drug formulations and successfully developed completely novel validated method for the same.

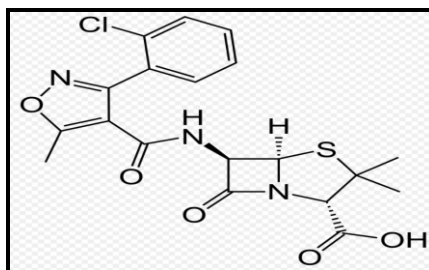
**OBJECTIVE**

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

## STRUCTURE



Cefixime



Cloxacillin

## MATERIAL AND METHODS

## Chemicals and Reagents

Standard Cefixime and Cloxacillin 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 Agilent 1200 series 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)

Gradient program:		
Time (in minutes)	% Mobile phase A	% Mobile phase B
0.01	100	0
4.0	0	100
4.1	100	0
6.0	100	0

## PREPARATION OF STANDARD SOLUTION

Weigh accurately 20 mg of Cefixime standard and 50 mg Cloxacillin standard 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 250 µg/mL of Cloxacillin 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.

## PREPARATION OF SAMPLE SOLUTION

Commercial brand containing of Cefixime and Cloxacillin in combination was procured. Each brand contained a label claim of 200 mg of Cefixime and 500 mg of Cloxacillin per tablet. Five tablets of each brand were weighed and powdered for the analysis. The

powder (1130 mg) equivalent to 200 mg of Cefixime and 500 mg of Cloxacillin 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 250 µg/mL of Cloxacillin.

ANALYTICAL METHOD VALIDATION<sup>[8-9]</sup>

## 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 CFX and CLX 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 Cloxacillin was observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 10 µg/mL to 200 µg/mL for Cefixime and 25 µg/mL to 500 µg/mL for Cloxacillin. The calibration curves were constructed with concentration (C) against peak area. The slope, intercept, regression equation and correlation coefficient for the CFX and CLX 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 Cloxacillin were found to be 7.5 µg/mL and 25.0 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 Cloxacillin at working level i.e. 100% both at intra-day (six times within 24 hour) and inter-day (six times 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 CFX and CLX. 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 Cloxacillin

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 Cloxacillin were measured and the amount of Cefixime and Cloxacillin 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 ( $2.0 \pm 0.2$  mL/min) and column oven temperature ( $40 \pm 2^\circ\text{C}$ ). One factor at the time was changed to estimate the effect. The solutions containing 100 µg/mL of Cefixime and 250 µg/mL of Cloxacillin 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 Cloxacillin are shown in (**Table.3**).

**Table 1: Method Validation Parameters for the Determination of Cefixime and Cloxacillin.**

Parameters	Values	
	Cefixime	Cloxacillin
System suitability		
Theoretical Plates-	More than 16874	More than 46547
Tailing Factor-	1.0	1.0
Linearity range (µg/mL)	10 to 200 µg/mL	25 to 500 µg/mL
Slope (m) <sup>a)</sup>	2818.5	621.85
Intercept(c) <sup>a)</sup>	645.19	605.08
Correlation coefficient (R <sup>2</sup> )	0.999	1.000
LOD (µg/mL)	3.0 µg/mL	7.5 µg/mL

<b>LOQ (<math>\mu\text{g/mL}</math>)</b>	10.0 $\mu\text{g/mL}$	25.0 $\mu\text{g/mL}$
<b>Intraday precision (n=6)</b>	0.3%	0.3%
<b>Interday precision (n=6)</b>	0.3%	0.3%
<b>Assay</b>	99.9% to 100.7%	98.7% to 99.4%
<b>Recovery</b>	98.5% to 99.5%	98.6% to 101.3%

**Sample Details**

Brand Name: ZIFI-LBX NEO (FDC Limited)

Batch No.: 590D003

API: Cefixime-200 mg and Cloxacillin-500 mg

Excipients: q.s.

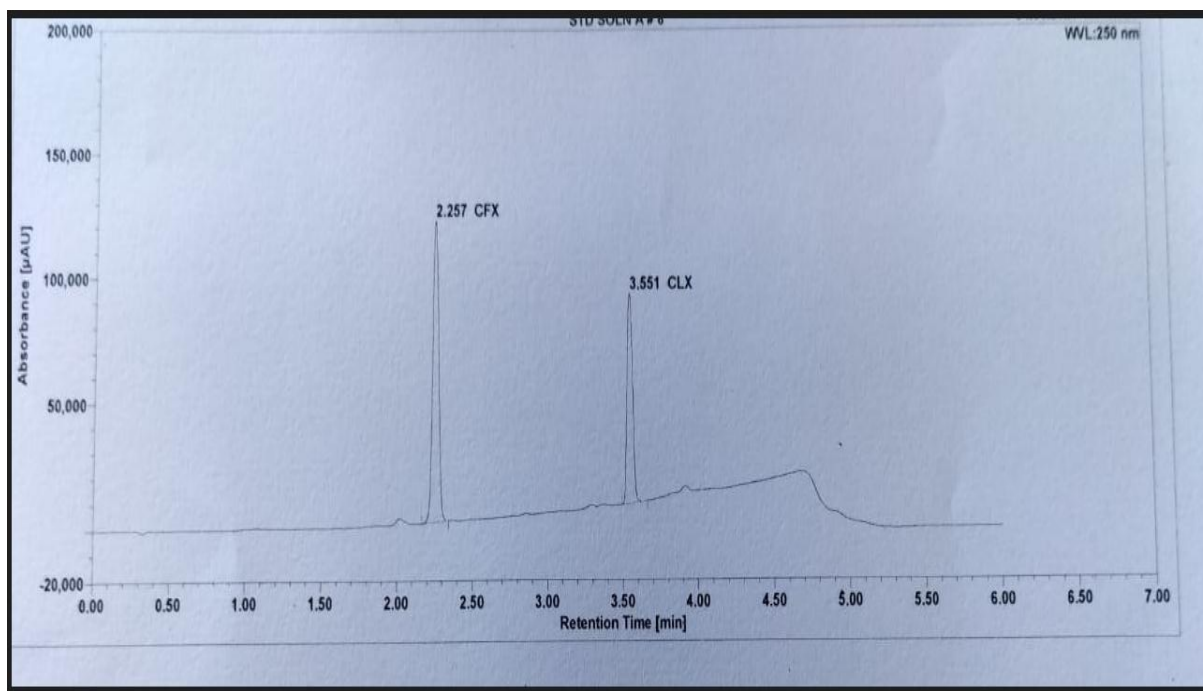
Colours: Lake of sunset Yellow FCF and Titanium dioxide

**Table 2: Result of Assay studies of Cefixime and Cloxacillin.**

Brand name	ZIFI-LBX NEO (FDC Limited)	
	Cefixime	Cloxacillin
<b>Labeled claim (mg)</b>	200mg	500mg
<b>Drug found in mg</b>	200.4 mg	495.3 mg
<b>% RSD (n=3)</b>	0.3	0.3
<b>% Assay</b>	100.2 %	99.1 %

**Table 3. Results of Recovery studies of Cefixime and Cloxacillin.**

Analyte	Level	Amount added for recovery study (in mg/mL)	Amount found in recovery study (in mg/mL)	RSD (%) (n = 3)	Recovery (%)	
					Minimum	Maximum
Cefixime	50%	50.0	49.80	0.2	99.2	99.5
	100%	100.0	99.30	0.3	98.6	99.3
	150%	150.0	148.35	0.3	98.5	99.1
<b>Range</b>					<b>98.5</b>	<b>99.5</b>
Cloxacillin	50%	125.0	50.60	0.9	99.6	101.3
	100%	250.0	100.6	0.4	99.4	100.1
	150%	375.0	151.8	0.5	98.6	99.5
<b>Range</b>					<b>98.6</b>	<b>101.3</b>

**Figure 1: Chromatogram for Cefixime and Cloxacillin.**

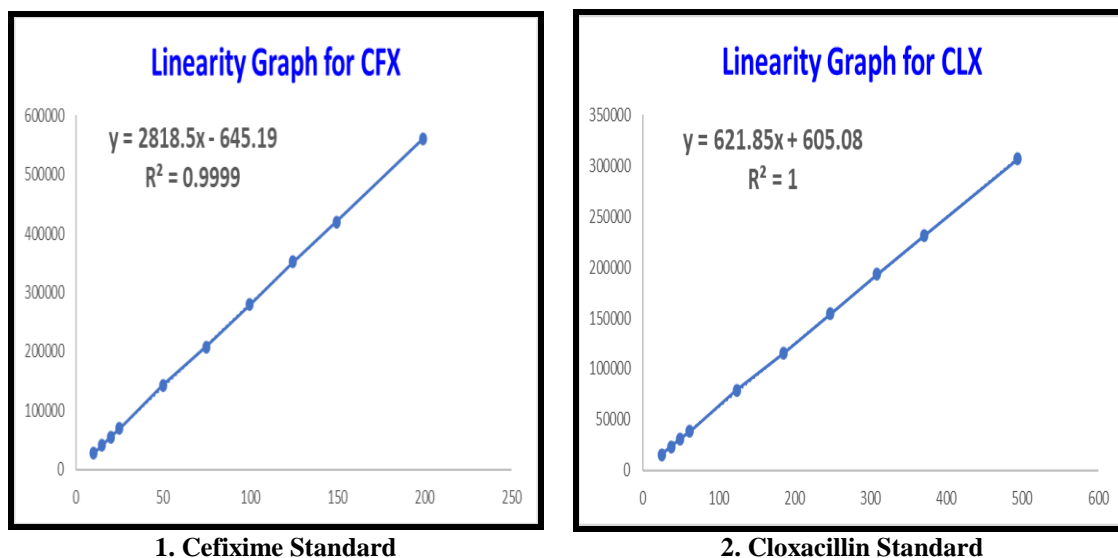


Figure 2: Linearity graph for.

Y-axis – Peak Area

X-axis- Concentration of Drug in µg/mL

## RESULT AND DISCUSSION

In the present work conditions were optimized for development and validation of a simple and accurate HPLC method for simultaneous quantification of Cefixime and Cloxacillin 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 CFX and CLX were obtained with gradient elution at retention time of 2.3 min and 3.6 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 Cloxacillin 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.

## 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 Cloxacillin in routine quality control analysis in combined drug formulations.

## ACKNOWLEDGEMENT

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