Development and Validation of Analytical Method for Simultaneous Estimation of Cefixime and Ofloxacin in Bulk and Tablet Dosage Form by RP-HPLC Method

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Abstract

The objective of present work was to develop and validate a simple, accurate, precise HPLC method for the estimation of cefixime and ofloxacin. The chromatographic separation was achieved on a Hypersil BDSC18 column (4.6x250 mm, 5µm particle size). Different mobile phase systems in different proportions were tried. For HPLC method a mobile phase consisting of Methanol and Water (70:30) produced symmetric peaks with good resolution for both the drugs. Next, the drugs were chromatographed under different flow rates from which a flow rate of 1.0 ml/min was selected. The retention times of cefixime and ofloxacin were found to be 2.96 min and 4.15 min, respectively. The proposed method was found to have excellent linearity in the concentration range of 20-80 mg/ml with correlation coefficient r² = 0.999 and 0.999 for cefixime and ofloxacin respectively. The method was validated for linearity, precision, LOD, LOQ and robustness. The proposed method optimized and validated as per ICH guidelines.

Keywords: Cefixime, Ofloxacin, Robustness.

1. INTRODUCTION

Cefixime and Ofloxacin are antibacterial drugs. Cefixime is chemically: (6R,7R)-7-{[12-(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. It is a III generation Cephalosporin antibiotic that acts by inhibiting cell wall synthesis. It has a molecular weight of 452.453g/Mo/l. Cefexime is a white to light yellow crystalline powder soluble in methanol. Ofloxacin is (9-flouro-2,3-dihydro-3-metyl-10(4-methyl-1-piparazinyl)7-oxo-7H-pyrido(1,2,3 de)1,benozxazine-6-carboxylic acid. Ofloxacin is a second generation flouroquinolone and it is soluble in water and methanol. 1-3

![Cefexime](image1)

![Ofloxacin](image2)

**Fig 1: Cefexime**

**Fig 2: Ofloxacin**

Literature survey revealed that several methods were reported for cefixime and ofloxacin individually and in combinations Dragica, 2003; Kathiresan, 2009; Neto et al., 2005; Aghazadeh, 2001; Kim, 2009; Shah, 2006; 2010; Krzysztof, 2001; Malathi, 2009; Dhoka, 2010; Timreyns, 2006; prabhu.s, 2010; Raj k, 2010; Rathinavel, 2008; Shah.j, 2010; s.low, 1989; s.s.Zade, 2013; B.thomas, 2010; Khaja, 2010; Deshpande et al., 2010; Nanda, 2009). Therefore, the main objective of this study was to attempt to develop a simple and rapid analytical method for simultaneous estimation of cefixime trihydrate and clavulanate potassium in a single dosage form and validate the proposed assay. 4-7

### 2. MATERIALS AND METHODS

**Apparatus:** The HPLC waters 2690/5 liquid chromatograph equipped with a PDA detector, the software installed was Empower, with 20µl loop, Hypersil-BDS C18 column (250mmx4.6mm, 5µl). The other instrument included are (SARTORIOUS) electronic balance and a sonicator (Fast clean).

**Chemicals and reagents**

Active pharma ingredient of cefixime and Ofloxacin was obtained as a gift sample from Arch Pharma Ltd, purified water HPLC grade was prepared by triple glass distillation and filtered through a 0.45µ membrane filter. Methanol HPLC grade was run at a flow rate of 1.0ml/min, 20µl of the sample was injected in the chromatographic system. Mobile phase comprising of Methanol: Water at the ratio (70:30). The column temperature was ambient with a detection wavelength of 290.

**Preparation of standard solution**

Stock solutions were prepared by dissolving 10mg cefixime of and 10mg of ofloxacin in mobile phase separately. Aliquots of standard solution of cefixime and ofloxacin were transferred into 10ml volumetric flasks and solutions were made up to the volume to yield concentrations of cefixime and ofloxacin. 8, 9

**Pharmaceutical formulation**

Formulation Mahacef, manufactured by mankind pharma was purchased from the local pharmacy in Hyderabad.

**Preparation of sample solution**

For analysis of commercial formulation, 20 tablets of mahacef of cefixime 200mg and ofloxacin 200mg were weighed the average weight was calculated and powdered. A quantity equivalent to 200mg of cefixime and 200mg of ofloxacin was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins. The solution was allowed to stand at a room temperature for 20-30mins and filter it through a
whatmann filter paper. Then suitable aliquots of formulation solution were prepared and injected into HPLC to obtain concentration in linearity range. 10-13

Validation of analytical method

ACCURACY: Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Recovery studies of the drug were carried out for determining accuracy parameter. Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out in 50% 100% and 150% levels. 14,15

PRECISION: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

a. System precision: Standard solution prepared as per test method and injected five times. 16

b. Method precision: Prepare five sample preparations individually using the single as per test method and injected each solution. 17,18

LINEARITY: The linearity of analytical method is the ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the given range. The linearity was performed by seven different concentrations, which cefexime and ofloxacin were injected and calibration curve were plotted. The linearity of was found to be in the range of 20-80µg/ml respectively. The chromatograms of the resulting solutions cefexime and ofloxacin were recorded. The plot showing linearity and range study for is shown in figure.

Plot of linearity and range study for clavulanic acid

Fig 3: Plot of linearity and range study for Cefixime

Fig 4: Plot of linearity and range study for Ofloxacin

Ruggedness:

a) System to System variability: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. A comparison of both the results obtained on two different HPLC systems, shows that the assay test method is rugged for System to system variables. 19

Robustness: The robustness of an analytical procedure are a measure of its capacity to remain unaffected by small, but deliberate changes in the method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated. 20

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD of an analytical procedure is the lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value where as LOQ is the lowest amount of analyte in
a sample which can be quantitatively determined with suitable precision and accuracy.

![Optimized chromatogram](image)

**Fig 5: Optimized chromatogram**

**Table 1: results of validation parameters of RP-HPLC**

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Validation Parameter</th>
<th>cefixime</th>
<th>ofloxacin</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Linearity (in µg)</td>
<td>20 – 80</td>
<td>20 – 80</td>
<td>Correlation coefficient (R²=0.999 or 0.999)</td>
</tr>
<tr>
<td>2.</td>
<td>Regression Line</td>
<td>y=63512x +79646</td>
<td>y=2107.4x+2621.2</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>R² Value</td>
<td>0.999</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Precision</td>
<td>System Precision (RSD) 0.523</td>
<td>0.455</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Method Precision(%RSD)</td>
<td>0.423</td>
<td>0.501</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>LOD</td>
<td>0.456</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>LOQ</td>
<td>3.508</td>
<td>4.92</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Assay of marketed formulation</td>
<td>99.1%</td>
<td>100.5%</td>
<td>95-105%</td>
</tr>
<tr>
<td>8.</td>
<td>% Recovery</td>
<td>98-101</td>
<td>98-101</td>
<td>95-105%</td>
</tr>
<tr>
<td>9.</td>
<td>Ruggedness</td>
<td>0.4</td>
<td>0.34</td>
<td>RSD&lt;2%</td>
</tr>
</tbody>
</table>

**3. RESULTS AND DISCUSSION**

- The slope, intercept and correlation coefficient values were found to be 63512, 79646 and 0.999 and 2107.4, 2621.2 and 0.999 for cefixime and ofloxacin respectively.
- The LOD of cefixime and ofloxacin were found to be 0.456 and 0.79µg/ml respectively. The LOQ of cefixime and ofloxacin found to be 3.508µg/ml and 4.92µg/ml respectively.
- Precision of the developed method was studied. Low % RSD values indicate that the method is precise. \(^{21-23}\)

**4. CONCLUSION**

The proposed RP-HPLC method for the estimation of the cefixime and ofloxacin in the pharmaceutical dosage form were simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved was good by RP-HPLC method. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms. In this proposed method symmetrical peaks with good resolution were obtained. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms. In this proposed method symmetrical peaks with good resolution were obtained.

**Table 2: Summary of analysis of cefixime and ofloxacin by RP-HPLC method**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Labeled amount, mg/tablet</th>
<th>Estimated Amount, mg/tablet</th>
<th>% Label claim</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEF</td>
<td>200</td>
<td>199.1</td>
<td>99.5</td>
<td>0.98</td>
</tr>
<tr>
<td>OFL</td>
<td>200</td>
<td>201</td>
<td>100.5</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**5. ACKNOWLEDGEMENT**

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**6. REFERENCES**

4. Sharif S, Khan IU, Ashfaq M, Iqbal MS, Ahmad S. Development and validation of a high performance liquid chromatographic method for the simultaneous determination of potassium clavulanate and cefadroxil in synthetically...

