Development and Validation of High Performance Liquid Chromatographic Method for Simultaneous Estimation of Potassium Clavulanate and Cefixime Trihydrate in Tablet Dosage Form.

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ABSTRACT

In the present study a simple, accurate and precise reverse phase liquid chromatographic method has been developed and validated for simultaneous estimation of Potassium Clavulanate and Cefixime Trihydrate from tablet dosage form. The method was developed using Waters HPLC system on a L1 column (Hypersil Gold: 250mm x 4.6 mm, 5µm) using a mixture of 0.0075 M Tetra Butyl Ammonium hydroxide solution of pH 6.8 and Methanol in the ratio 80:20 v/v as mobile phase in an isocratic elution mode at a flow rate of 1.0 ml/min, at 40ºC with a load of 20µl. The detection was carried out at 230 nm. The retention time of Potassium Clavulanate and Cefixime were found to be around 4.63 min and 11.89 min respectively. The method was validated with respect to linearity, robustness, precision and accuracy. The proposed method was successfully applied for the simultaneous quantitative determination of Potassium Clavulanate and Cefixime from the tablet dosage form.

Key words: Potassium Clavulanate, Cefixime Trihydrate, Liquid Chromatography.

INTRODUCTION

Potassium Clavulanate is (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptan-2-carboxylate.\(\text{Clavulanate is a potent inhibitor of the beta-lactamase produced by Staphylococcus aureus. Clavulanic Acid enhances the activity of penicillin and cephalosporin antibiotics against many resistant strains of bacteria.}\)\(^{1}\) While Cefixime is \(\text{(8R,7R)-7-[[2(Z)-2-[(2-aminothiazol-4-yl)-[carboxamidothoxy] imino] acetyl] aminol-3-ethynyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid.}\)\(^{1}\)

It is generally classified as third generation cephalosporin antibiotic and is given by mouth in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory tract infections like bronchitis and urinary tract infections.\(^{1}\) Literature survey reveals separate HPLC method of analysis for Potassium Clavulanate in bulk as well as in combination with other drugs like Amoxycillin from tablet is available.\(^{2,5-14}\) Furthermore HPLC assay methods for Cefixime Trihydrate in bulk, oral suspension and tablets dosage forms\(^{15,16}\) or in combination with other drugs are available.\(^{16}\) Spectrophotometric method of analysis of Cefixime in combination with Ornidazole has also been reported.\(^{17}\) Several methods also have been reported for simultaneous determination of Potassium Clavulanate and Cefixime Trihydrate by HPLC.\(^{18,19}\) The objective of this work is to develop an accurate, specific, repeatable and validated HPLC method for simultaneous determination of Potassium Clavulanate and Cefixime from tablet dosage form. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines.\(^{19}\)

MATERIALS AND METHODS

Materials

Methanol used was of HPLC grade of Merck and Milli Q water was used for the preparation of mobile phase. All the other reagents like 0.1 (N) Tetra Butyl Ammonium Hydroxide solution in Isopropanol used were of AR/GR grade. All the glass wares used were of standard quality.

Drugs used

Diluted Potassium Clavulanate (Assay: 51.68 w/w, H:\(\text{O: 1.26 w/w)}\) and Cefixime (Assay: 99.2% w/w, H:\(\text{O: 11.3% w/w)}\) reference standards of Central Drugs Laboratory were used. Tablet formulation containing Potassium Clavulanate equivalent to Clavulanic Acid 125 mg and Cefixime Trihydrate equivalent to Cefixime 200 mg was used as the sample during the method development process.

Instrumentation

An Isocratic Waters HPLC with a 515 pump, 2487 dual UV-Visible detector and L1 column (Hypersil Gold: 250mm x 4.6mm, 5µm) were used for the analysis. The HPLC system was well equipped with Empower 2 software for data processing.

Chromatographic Condition

The mixture of 0.0075 M Tetra Butyl Ammonium Hydroxide solution of pH 6.8 (adjusted with 10% H:\(\text{PO4/ NH4OH)}\) and Methanol in the ratio 80:20 v/v was used as mobile phase. The Tetra Butyl Ammonium Hydroxide solution was filtered through 0.22 micron membrane prior to mix with Methanol. The mobile phase was ultrasonicated for 5 minutes to degas the mixture and then used. The separation was achieved on a L1 column (Hypersil Gold: 250 mm x 4.6mm, 5µm). The flow rate of 1.0 ml/min was set for the isocratic elution and detection was carried out at 230 nm. All determinations were performed at a constant column temperature of 40ºC on a load of 20µl. Mixture of Methanol and water in the ratio 60:40 was used as diluent for the preparation of sample and standard solutions. The summary of chromatographic condition is given in Table 1.

Table 1: Summary of chromatographic condition:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Hypersil Gold L1 (4.6 x 250 mm, 5µm)</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>0.0075 M Tetra butyl Ammonium Hydroxide pH8. Methanol 20: 80 v/v</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0 ml/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>40ºC</td>
</tr>
<tr>
<td>Detection Wavelength</td>
<td>230 nm</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>20 µl</td>
</tr>
<tr>
<td>Diluent</td>
<td>Methanol: Water = 60:40</td>
</tr>
</tbody>
</table>

Solutions Preparation

A mixed standard solution was prepared by dissolving 27.9 mg of diluted Potassium Clavulanate and 24.2 mg Cefixime Trihydrate in 100 ml diluents in order to obtain a working concentration of Clavulanic Acid around 125 mcg/ml and Cefixime 200 mcg/ml.

RESULTS AND DISCUSSION

Method Development

To optimize HPLC parameters different compositions of solvents containing mixtures (v/v) of 0.0075 (M) Tetra Butyl Ammonium Hydroxide solution and Methanol at several pH values were tried as mobile phase. Elution of mixed standard solution was also done under varied flow rates and also monitored at different wavelengths like 225 nm, 230 nm and 235 nm. Finally the suitability of the mobile phase was decided on the basis of ease of preparation, shapes and resolution of peaks and convenient analysis time. The mixture of 0.0075 (M) Tetra Butyl Ammonium Hydroxide solution of pH 6.8 and Methanol in the ratio 80:20 v/v was found to be most suitable for obtaining well defined and well resolved peaks of Clavulanic Acid and Cefixime at a flow rate of 1.0 ml/min at 40ºC on a L1 column. 230 nm was selected as the optimum wavelength for detection and quantitation, at which the best detector response for both Clavulanic Acid and Cefixime were obtained. The mean retention time and standard deviation for Clavulanic Acid and Cefixime were found to be 4.63 ± 0.031 min and 11.89 ± 0.044 min.

Tablet Assay

Twenty tablets were weighed and powdered. An accurately weighed quantity of tablet powder equivalent to about 12.5 mg Clavulanic Acid and 20 mg Cefixime was transferred to 100 ml volumetric flask and 70 ml of diulents was added to it. The solution was sonicated for 10-15 minutes. Finally the volume was made up to the 100 ml mark with the diluent. Solution was mixed thoroughly, filtered through 0.22 µm membrane filter and analyzed under optimized chromatographic condition. A representative chromatogram of mixed standard solution of Clavulanic Acid (~120 mcg/ml) and Cefixime (~200 mcg/ml) is given in Figure 1. The average result of six estimations is given in Table 2.

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**MEHOD VALIDATION**

**Linear**

The linearity of the method is the ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The linearity study was made from a series of standard solutions of Potassium Clavulanate and Cefixime Trihydrate. For Potassium Clavulanate suitable volumes of stock solution of 433 mcg/ml was diluted to obtain a series of solutions having concentration of 43, 65, 87, 108, 130 and 173 mcg/ml of Clavulanic Acid. For Cefixime Trihydrate a standard stock solution of 510 mcg/ml was diluted in order to obtain a series of solutions having concentration of 51, 102, 153, 204, 255, 306 and 357 mcg/ml of Cefixime. Each solution was injected in replicate and chromatograms were recorded. The average peak areas were plotted against concentration to obtain calibration curves for Clavulanic Acid and Cefixime. The Calibration curves were linear in the range 10-180 mcg/ml for Clavulanic Acid and 10-360 mcg/ml for Cefixime. The calibration curves for Clavulanic Acid and Cefixime are shown in Figure 2 & Figure 3 and Linear Regression Analysis results are summarized in Table 3.

**Precision**

Precision study was assessed by injection repeatability and sample repeatability tests. For injection repeatability mixed standard solution of Clavulanic Acid and Cefixime was injected in replicate. For sample repeatability study, six sample solutions of tablet were prepared following the method described under Tablet Assay and assayed. The % RSD of the six separate assay results was calculated. Injection repeatability was confirmed from the low %RSD values of peak area for both the components. The % RSD for assay results of six determinations was less than 1% for both the components, which confirms the high degree of precision of the method.

**Robustness**

To evaluate the robustness, the developed method was subjected to small deliberate variations in the optimized method parameters like variation of flow rate ±1 ml/min (i.e. 0.9 ml/min, 1.0 ml/min and 1.10 ml/min) and detection wavelength i.e. 230±1 nm. The mixed standard solution containing 105.4 mcg/ml Clavulanic Acid and 207.7 mcg/ml Cefixime was injected in replicate under varied chromatographic conditions and the standard deviation of the retention time of each analyte were calculated. The method was found to be robust as the slight deliberate variations in detection wavelength and flow rate did not lead to changes in retention times of peak of interest. While evaluating the robustness data it was observed that system suitability parameters (e.g. Tailing Factor, Plate counts, Resolutions etc) were found to be within the specified limits under those deliberately varied conditions, which ensures that the validity of the analytical procedure was maintained whenever used. The result of robustness study is summarized below in Table 4.

**Accuracy (Recovery Study)**

The accuracy was evaluated from the recovery study at three different levels. It was carried out by spiking standard of Potassium Clavulanate and Cefixime Trihydrate to the pre-analyzed sample at three different levels i.e. 80%, 100% and 120% of the amount of each component contributed from the tablet powder. Each solution was injected in triplicate. The amount of drug recovered is calculated in each case. The % recovery was also calculated by using the following formula = (Amount of drug recovered/ Amount of drug added) x 100. The recovery of the added standard was studied at three different levels and the result is given in Table 5.

**LOD and LOQ Determination**

Limit of detection can be calculated by using following formula:

\[
\text{LOD} = \frac{3.3 \cdot S}{\sigma}
\]

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

\[
\text{LOQ} = 10 \cdot S
\]

Where \( \sigma \) = Standard deviation of the Y intercept of regression line \[14\]

\( S \) = Slope of the calibration curve

The Limit of Detection (LOD) were found to be 1.55 mcg/ml for Clavulanic Acid and 4.5 mcg/ml for Cefixime while Limit of Quantitation (LOQ) were found to be 4.7 mcg/ml for Clavulanic Acid and 13.63 mcg/ml for Cefixime.

**System Suitability Testing**

System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability was assessed by injecting Clavulanic Acid and Cefixime mixed standard preparation in replicate. Parameters such as theoretical plates, tailing factor, resolution were determined. The System suitability parameters for the method are listed below in the Table 6.
CONCLUSION
The reported RP-HPLC method was proved to be simple, rapid and reproducible. The validation data indicate good precision, accuracy and reliability of the method. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in simultaneous determination of Potassium Clavulanate and Cefixime Trihydrate in tablet dosage forms.

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Table 6: System Suitability Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Clavulanic Acid</th>
<th>Cefixime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration Range (mcg/ml)</td>
<td>10-180</td>
<td>10-360</td>
</tr>
<tr>
<td>Retention Time (min)</td>
<td>4.63</td>
<td>11.89</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>11000</td>
<td>8000</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>0.98</td>
<td>1.23</td>
</tr>
<tr>
<td>Resolution</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>LOD (mcg/ml)</td>
<td>1.55</td>
<td>4.5</td>
</tr>
<tr>
<td>LOQ (mcg/ml)</td>
<td>4.7</td>
<td>13.63</td>
</tr>
</tbody>
</table>

REFERENCES
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