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 2487 Dual UV-Visible Detector 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]heptanes-2-carboxylate.[1]Clavulanic Acid is a potent inhibitor of the beta-lactamase produced by Staphylococcus aureus. Clavulanic Acid enhances the activity of penicillin and cephalosporin antibacterials against many resistant strains of bacteria.[2,3] While Cefixime is (6R,7R)-7-[(2Z)-2-(2-aminothiazol-4-yl)[(carboxymethoxy)imino]acyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid.[4,5] It is generally classified as third generation cephalosporin antibacterial 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.[6] Literature survey reveals separate HPLC method for analysis of Potassium Clavulanate in bulk as well as in combination with other drugs like Amoxicillin from tablet is available.[7,8,9] Furthermore HPLC assay methods for Cefixime Trihydrate in bulk, oral suspension and tablets dosage forms[10,11,12] or in combination with other drugs are available.[13] Spectrophotometric method of analysis of Cefixime in combination with Omdizole has also been reported.[14] Several methods also have been reported for simultaneous determination of Potassium Clavulanate and Cefixime Trihydrate by HPLC.[11,12] 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.[15]

MATERIALS AND METHODS

Materials

Potassium Clavulanate and Cefixime Trihydrate from the tablet dosage form. The method was developed using Waters 2487 Dual UV-Visible Detector with a load of 20µl. Mixture of Methanol and water in the ratio 60:40 was used as diluent 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 4.6 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 40°C with a load of 20µl. 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 40°C 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 was 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.

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 40°C on a L1 column.

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% H3PO4/ 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 filter.

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 diethylether in order to obtain a working concentration of Clavulanic Acid around 125 mcg/ml and Cefixime 200 mcg/ml.

Spectrophotometric method of analysis of Cefixime in combination with

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MEHOD VALIDATION

Linearity

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 Clavulanate. 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 Clavulanate Acid and Cefixime. The Calibration curves were linear in the range 10-180 mcg/ml for Clavulanate Acid and 10-360 mcg/ml for Cefixime. The calibration curves for Clavulanate Acid and Cefixime are shown in Figure 2 & Figure 3 and Linear Regression Analysis results are summarized in Table 3.

LOD and LOQ Determination

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated by using the following formulae:

\[ \text{LOD} = \frac{3 \times \sigma}{S} \]
\[ \text{LOQ} = \frac{10 \times \sigma}{S} \]

where \( \sigma \) is the standard deviation of the Y intercept of the regression line, \( S \) is the slope of the calibration curve.

System Suitability Testing

The system suitability testing was carried out to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability was assessed by injecting Clavulanate 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount present (mg)</th>
<th>Amount Found (mg)</th>
<th>% Estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clavulanate Acid</td>
<td>125 mg</td>
<td>115.71 mg</td>
<td>92.57%</td>
</tr>
<tr>
<td>Cefixime</td>
<td>200 mg</td>
<td>199.55 mg</td>
<td>99.78%</td>
</tr>
</tbody>
</table>

Table 2: Result of HPLC Assay

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:

\[ \text{Recovery} = \frac{\text{Amount of drug recovered}}{\text{Amount of drug added}} \times 100 \]

The % recovery 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 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 Clavulanate 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 was 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.

Table 4: Robustness Data in terms of Retention Time (mean ± S.D.)

```
<table>
<thead>
<tr>
<th>Level</th>
<th>Wavelength (nm)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clavulanate Acid</td>
<td>Cefixime</td>
<td>Clavulanate Acid</td>
</tr>
<tr>
<td>80%</td>
<td>5.04 (4.98, 5.10)</td>
<td>1.12 (1.10, 1.14)</td>
</tr>
<tr>
<td>100%</td>
<td>5.04 (4.98, 5.10)</td>
<td>1.12 (1.10, 1.14)</td>
</tr>
<tr>
<td>120%</td>
<td>5.04 (4.98, 5.10)</td>
<td>1.12 (1.10, 1.14)</td>
</tr>
</tbody>
</table>
```

Table 5: Accuracy Results

```
<table>
<thead>
<tr>
<th>Level</th>
<th>Clavulanate Acid</th>
<th>Cefixime</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>0.99 (0.97, 1.01)</td>
<td>0.99 (0.97, 1.01)</td>
</tr>
<tr>
<td>100%</td>
<td>1.00 (0.98, 1.02)</td>
<td>1.00 (0.98, 1.02)</td>
</tr>
<tr>
<td>120%</td>
<td>1.01 (0.99, 1.03)</td>
<td>1.01 (0.99, 1.03)</td>
</tr>
</tbody>
</table>
```

Table 3: Linear Regression Data

```
Parameter  | Clavulanate Acid | Cefixime |
-----------|-----------------|----------|
Concentration Range (mcg/ml) | 10-180 | 10-360 |
Slope           | 18800 | 40600 |
Intercept       | 98400 | 42900 |
R²              | 0.9999 | 0.9998 |
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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>10000</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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