REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF CEFUROXIME AXETIL AND POTASSIUM CLAVULANATE IN TABLET DOSAGE FORM

Mahima R. Sengar, Santosh V. Gandhi *, Upasana P. Patil, Vivek S. Rajmane

Department of Pharmaceutical Analysis,
A.I.S.S.M.S. College of Pharmacy, Kennedy Road, Near R.T.O.,
Pune - 411 001. India.

*Corres.author:santoshvgandhi@rediffmail.com

ABSTRACT: A simple, specific, accurate, and precise reverse phase high-performance liquid chromatographic method for analysis of Cefuroxime axetil and Potassium clavulanate has been developed. Separation of drugs was carried out on Jasco HPLC system with Hypersil Gold C-18 column (250 mm × 4.6 mm i.d.), using 0.01M Potassium dihydrogen phosphate: methanol 60: 40 (v/v) as mobile phase. Quantitation of drugs was carried out at wavelength 225 nm. Results were found to be linear in the concentration range of 5-50 µg/ml for CA and 5-30 µg/ml for PC. Mean retention times for Potassium clavulanate (PC) and the two isomers of Cefuroxime axetil (CA1 and CA2) were found to be 2.573, 8.293 and 9.987 min, respectively. Intra-day variation, as RSD (%), was 0.328 for Cefuroxime axetil and 0.382 for Potassium clavulanate. Interday variation, as RSD (%) was 0.545 for Cefuroxime axetil and 0.552 for Potassium clavulanate. The % assay was found to be 100.976 ± 0.439 for Cefuroxime axetil and 101.053 ± 0.423 for Potassium clavulanate (Mean ± S.D., n = 6).

KEYWORDS: RP-High performance liquid chromatography, Cefuroxime axetil, Potassium clavulanate

INTRODUCTION

Cefuroxime Axetil (CA), (RS)-1 hydroxyethyl (6R,7R)-7-[2-(2-furyl) glyoxyl-amido] -3- (hydroxyl methyl -8-oxo-5- thia-1- azabicyclo[4.2.0]-oct-2-ene-2-carboxylate,7-(Z)-(O-methyl-oxime),1-acetate3-carbamate ) is second generation cephalosporin used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. Clavulanic acid administered as potassium salt, is a powerful inhibitor of β-lactamase enzyme and is most often formulated in combination with antibiotics for treatment of infection caused by lactamase producing bacteria.

*Corres.author: Gandhi Santosh V.
AISSMS College of Pharmacy, Kennedy road, Near R.T.O., Pune. 411 001 Maharashtra, India.
Ph. No. +91-20-26058204, +91-9422349792
E-mail: santoshvgandhi@rediffmail.com

EXPERIMENTAL

CHEMICALS AND REAGENTS

Potassium dihydrogen orthophosphate ( AR grade, S.d. fine - Chem Laboratories Pvt. Ltd., Mumbai, India), Methanol (HPLC grade, Merck Specialities Pvt.
Twenty tablets were weighed accurately; the average weight was determined and then ground to a fine powder. Powder equivalent to 10 mg of PC was weighed; transferred to a 10 ml volumetric flask containing about 5 ml of mobile phase and sonicated for 5 min. Then volume was made up to the mark with the mobile phase; filtered through Whatman filter paper no. 41. From this solution 0.1 ml was taken and transferred to 10 ml volumetric flask. This sample solution having concentration 10 µg/ml of PC was injected and chromatogram was obtained. The injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in Fig. 1.

### RECOVERY STUDIES
To study the accuracy and precision of the proposed method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50 %, 100 % and 150 %.

### ROBUSTNESS
Robustness of the developed method was determined by small but deliberate changes in chromatographic conditions such as flow rate (± 0.02 ml/min), wavelength (± 1 nm), and mobile phase composition (± 2 %). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

### RESULTS AND DISCUSSION
For RP-HPLC method different mobile phases were tried and the mobile phase containing 0.01 M Potassium dihydrogen orthophosphate and methanol in ratio 60:40 (% v/v) was found to be optimal for obtaining well defined and resolved peaks with retention time 2.573 ± 0.047 min for PC, 8.273 ± 0.055 min for CA₁ and 9.987 ± 0.056 min for CA₂ (mean ± S.D.). The calibration plots were found to be linear over the ranges 5–50 µg/ml and 5–30 µg/ml for CA and PC, respectively, with correlation coefficients of 0.995 ± 0.332 and 0.991 ± 0.095 respectively. System suitability parameters for RP-HPLC method are listed in Table 1.

The LOD and the LOQ for the CA were found to be 2.409 and 7.951 µg/ml, respectively, and for PC were found to be 0.538 and 1.778 µg/ml, respectively. The % recovery was found to be 100.598 ± 0.371 for CA and 100.741 ± 0.418 for PC (Mean ± % RSD, n = 6). Results of recovery studies are represented in Table 2. The % RSD values were satisfactorily low indicating reproducibility of the method. The % assay was found to be 100.976 ± 0.439 for Cefuroxime axetil and 101.053 ± 0.423 for Potassium clavulanate (Mean ± S.D., n = 6).

### CONCLUSION
This work describes a simple, accurate and sensitive validated RP-HPLC method for simultaneous determination of both the drugs and method can be used conveniently for quality control purposes.
Table 1: System suitability parameters for RP-HPLC method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC</th>
<th>CA₁</th>
<th>CA₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates</td>
<td>4020</td>
<td>4071</td>
<td>4502</td>
</tr>
<tr>
<td>Asymmetry Factor</td>
<td>1.26</td>
<td>1.15</td>
<td>1.11</td>
</tr>
<tr>
<td>HETP (cm)</td>
<td>0.0062</td>
<td>0.0061</td>
<td>0.0055</td>
</tr>
<tr>
<td>Resolution</td>
<td>_</td>
<td>2.07</td>
<td>2.128</td>
</tr>
</tbody>
</table>

* With respect to previous peak.

Table 2: Recovery studies of CA and PC

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken,(μg/ml)</th>
<th>Amount added,(μg/ml)</th>
<th>Total amount found (μg/ml)</th>
<th>%Recovery*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>20</td>
<td>10</td>
<td>30.068</td>
<td>100.23</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20</td>
<td>40.464</td>
<td>101.16</td>
<td>0.408</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30</td>
<td>50.205</td>
<td>100.41</td>
<td>0.394</td>
</tr>
<tr>
<td>PC</td>
<td>10</td>
<td>05</td>
<td>15.062</td>
<td>100.41</td>
<td>0.386</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>20.070</td>
<td>100.35</td>
<td>0.474</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>15</td>
<td>25.364</td>
<td>101.46</td>
<td>0.396</td>
</tr>
</tbody>
</table>

* Average of three determinations.

Figure 1: Chromatogram of standard mixture containing PC 10 μg/ml (2.573 min) and CA 20 μg/ml (CA₁ 8.293 min; CA₂ 9.987 min).
ACKNOWLEDGEMENTS
The authors express their gratitude to Maxim Pharmaceuticals Pvt. Ltd. (Pune, India) and Medreich Pharmaceuticals Pvt. Ltd. (Bangalore, India) for the gift samples of pure drug. The authors thank Dr. K. G. Bothara, Principal, AESMS College of Pharmacy, Pune University, India, for providing research facilities.

REFERENCES
6) Krzekand J., Browska-Tylka M.D., Simultaneous determination of cefuroxime axetil and cefuroxime in pharmaceutical preparations by thin-layer chromatography and densitometry, Chromatographia, 2003, 58, 231.
15) ICH harmonised tripartite guideline, Q2 (R1), Validation of analytical procedures: Text and Methodology, Nov, 2005

*****