Simultaneous determination of cefuroxime axetil and ornidazole in tablet dosage form using reversed-phase high performance liquid chromatography*

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Abstract A simple accurate and sensitive reversed-phase high performance liquid chromatographic RP-HPLC method for the simultaneous determination of cefuroxime axetil and ornidazole in combined tablet dosage form has been developed. The method was performed with a HiQ-SIL C18 column 250 mm x 4.6 mm[1] and photodiode array PDA detector using 0.01 mol/L potassium dihydrogen orthophosphate-methanol[56:44] v/v as the mobile phase and tinidazole as the internal standard. Beer’s law obeys in the concentration ranges of 5 – 25 μg/mL and 10 – 50 μg/mL for cefuroxime axetil and ornidazole[6] respectively. The method has been successfully validated statistically and applied for the analysis of the drugs in pharmaceutical formulation.

Key words reversed-phase high performance liquid chromatography RP-HPLC cefuroxime axetil ornidazole

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Cefuroxime axetil CA R S-1-hydroxyethyl 6R 7R 2-furyl glyoxyl-amido-3-hydroxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate[7] Z O-methyl-oxime 1-acetate 3-carbamate is the second generation of cephalosporin used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria[8] whereas ornidazole OZ 1 3-chloro-2-hydroxypropyl[2]-methyl-5-nitroimidazole is used to cure protozoan infections[9]. Their structures are shown in Fig. 1.

![Fig. 1 Structures of CA and OZ](image)

Literature survey reveals spectrophotometric[10] and high performance thin-layer chromatographic HPTLC methods[11-15] for CA determination in combination with other drugs. Stability indicating[16] and bioanalytical chromatographic methods[15-18] for quantification of CA are also reported. Cefuroxime axetil is official in The United States Pharmacopeia USP which dictates reversed-phase high performance liquid chromatographic RP-HPLC method for CA determination as a single drug[19]. For simultaneous determination of OZ with other drugs[10-12] and HPTLC[11,13-14] methods have been reported. Bioanalytical chromatographic method[15] for OZ determination is also available. No reports were found for the simultaneous determination of CA and OZ by RP-HPLC method. Aim of the present work was to develop a simple economical rapid precise and accurate method for the simultaneous determination of binary drug formulation.

1 Experimental

1.1 Chemicals and reagents

Analytically pure sample of CA was kindly supplied by Maxim Pharmaceuticals Pvt. Ltd. Pune India whereas OZ and tinidazole TZ were kindly supplied by Cipla Ltd. Mumbai India and were used as such without further purifi-
cation. The pharmaceutical dosage form used in this study was the CEFAKIND-OZ 250 tablets manufactured by Akums Drugs & Pharmaceuticals Ltd. at Haridwar, India labeled to contain 250 mg of CA and 500 mg of OZ.

Potassium dihydrogen orthophosphate AR grade S. D. Fine-Chem Laboratories Pvt. Ltd. Mumbai, India methanol HPLC grade Merck Specialities Pvt. Ltd. Mumbai, India and water HPLC grade Loba Chemie Mumbai, India were used in the analysis.

1.2 Instruments and chromatographic conditions

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pump MD 2010 photodiode array PDA detector and Borwin-PDA Version 1.50 software was used. The separation was carried out on a HiQ-Sil C18 column 250 mm x 4.6 mm using 0.01 mol/L potassium dihydrogen orthophosphate-methanol 56:44 v/v as the mobile phase and TZ as the internal standard at a flow rate of 1.5 mL/min. Samples were injected using a Rheodyne injector with 20 µL loop. The detection wavelength was 281 nm.

1.3 Procedure

1.3.1 Preparation of standard stock solution

The standard stock solutions were prepared by dissolving 50 mg of CA and TZ separately in 50 mL of methanol to get a concentration of 1 mg/mL each and 50 mg of OZ in 25 mL of methanol to get a concentration of 2 mg/mL. They were further diluted 10 times with mobile phase to get working standard solutions of CA and TZ both having concentrations of 100 µg/mL and of OZ having a concentration of 200 µg/mL.

1.3.2 Preparation of calibration curve

Aliquots of working standard solution of CA 100 µg/mL and OZ 200 µg/mL were transferred in separate 10 mL volumetric flasks. In each flask 1 mL stock solution of TZ 100 µg/mL was added and the volume was made up to the mark with the mobile phase. Each solution having concentrations of 5 10 15 20 25 100 200 300 400 500 µg/mL of OZ was injected and the chromatograms were recorded. The peak area ratios of CA CA1 + CA2 to TZ and OZ to TZ were calculated and the respective calibration curves were plotted of the response factor against the concentration of drug. Limit of detection LOD and limit of quantification LOQ were calculated as 3.3 σ/S and 10 σ/S respectively as per ICH guidelines where σ is the standard deviation of the response y-intercept and S is the slope of the calibration plot.

1.3.3 Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately and ground to a fine powder. The powder equivalent to 50 mg of OZ, 25 mg of CA was weighed and transferred to a 25 mL volumetric flask containing about 20 mL of mobile phase and sonicated for 5 min. Then the volume was made up to the mark with the mobile phase filtered through Whatman filter paper No. 41 and 1 mL of this solution was transferred to a 10 mL volumetric flask. One milliliter of the working standard solution of TZ was added and the volume was made up to the mark with the mobile phase. This sample solution having concentrations 10 µg/mL of CA 20 µg/mL of OZ and 10 µg/mL of TZ was injected and the chromatogram was obtained. The injections were repeated six times and the peak areas were recorded. A representative chromatogram is given in Fig. 2.

![Fig. 2 Chromatogram of TZ 10 µg/mL, OZ 20 µg/mL and CA 10 µg/mL](image-url)

The peak area ratio of each of the drug to the internal standard was calculated and the amount of each drug present per tablet was estimated from the respective calibration curve. For the determination of CA the peak areas of both isomers CA1 and CA2 were added together and then the ratio to internal standard was calculated.

1.4 Recovery studies

To study the accuracy and precision of the proposed method recovery studies were carried out by the addition of standard drug solution to the preanalyzed sample solution at three different concentrations.
levels of 50% - 100% and 150%.

1.5 Robustness

The Robustness of the developed method was determined by the small but deliberate changes in chromatographic conditions such as the flow rate $\pm 0.02$ mL/min and wavelength $\pm 1$ nm and mobile phase composition $\pm 2\%$. It was observed that there were no marked changes in the chromatograms which demonstrated that the RP-HPLC method developed is robust.

2 Results and discussion

For RP-HPLC method different mobile phases were tried and the mobile phase containing 0.01 mol/L potassium dihydrogen orthophosphate and methanol in the ratio of 56:44 v/v was found to be optimal for obtaining well defined and resolved peaks with the mean retention times $\pm$ S. D. of $\bar{\tau} = 2.827 \pm 0.043$ min for TZA, $\bar{\tau} = 4.373 \pm 0.047$ min for OZ and $\bar{\tau} = 8.920 \pm 0.056$ min for CA.

Straight-line calibration graphs were obtained for CA and OZ with the method. Responses were found to be linear in the concentration ranges 5 – 25 $\mu$g/mL for CA and 10 – 50 $\mu$g/mL for OZ with correlation coefficient greater than 0.9989. LOD and LOQ were found to be 0.748 $\mu$g/mL and 2.267 $\mu$g/mL for CA and 1.026 $\mu$g/mL and 3.110 $\mu$g/mL for OZ respectively. System suitability parameters for the method are listed in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>System suitability parameters for RP-HPLC method</th>
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<tbody>
<tr>
<td>Parameter</td>
<td>TZA</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>7985</td>
</tr>
<tr>
<td>Asymmetry factor</td>
<td>1.45</td>
</tr>
<tr>
<td>HETP/cm</td>
<td>0.0031</td>
</tr>
<tr>
<td>Resolution</td>
<td>–</td>
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</tbody>
</table>

1$\%$ height equivalent of theoretical plate. 2$\%$ with respect to the previous peak.

The method was also evaluated by the assay of commercially available tablets containing CA and OZ. The percentage of labelled amount was found to be 101.04% with the relative standard deviation RSD of 0.452% for CA and 99.02% with RSD of 0.404% for OZ $n = 6$.

The results of recovery studies were shown in Table 2. The recoveries were close to 100% and the RSD values were satisfactorily low indicating that the method is reproducible.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Recovery studies of CA and OZ $n = 3$</th>
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<tbody>
<tr>
<td>Drug</td>
<td>Background/Added/Found/Recovery/RSD/</td>
</tr>
<tr>
<td></td>
<td>$\mu$g/mL</td>
</tr>
<tr>
<td>CA</td>
<td>10</td>
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<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>OZ</td>
<td>10</td>
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3 Conclusions

This work describes a simple accurate and sensitive validated RP-HPLC method for the simultaneous determination of both the drugs and the method can be used conveniently for quality control purposes.

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References