DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND LINEZOLID IN ITS COMBINED TABLET DOSAGE FORM

CHETANA RIBADIYA*1, HEMANG RIBADIA2, NIMISH TALAVIYA3, CHANDANI JOSHI4, ASHOK PARMAR5

ABSTRACT
A simple, precise, accurate and reproducible spectrophotometric method has been developed for simultaneous estimation of Cefixime Trihydrate (CEF) and Linezolid (LNZ) by employing first order derivative zero crossing method in Methanol. The first order derivative absorption at 290 nm (zero cross point of CEF) was used for quantification of Linezolid and 228 nm (zero cross point of LNZ) for quantification of Cefixime Trihydrate. The linearity was established over the concentration range of 2-18 µg/ml and 7-15 µg/ml for Cefixime and Linezolid with correlation coefficient r² 0.9970 and 0.9982, respectively. The mean % recoveries were found to be in the range of 98.36 % – 99.45 % and 100.10 % – 101.66 % for Cefixime and Linezolid, respectively. The proposed method has been validated as per ICH guideline and successfully applied to the estimation of Cefixime and Linezolid in bulk and in market formulation.

KEYWORDS
Cefixime Trihydrate, Linezolid, First Order Derivative Method, Methanol.

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INTRODUCTION

Cefixime Trihydrate (CEF) is an oral third generation cephalosporin antibiotic. Cefixime (C_{16}H_{15}N_{5}O_{7}S_{2}, 3H_{2}O), chemically, it is (6R,7R)-7-[(2- (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 trihydrate [1], clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinarytract infections [2,3].

Linezolid (LNZ) is a synthetic antibacterial agent of the oxazolidinone class of antibiotics. Linezolid is chemically N-[(5S)-3-[3-fluoro-4- (morpholin-4-yl) phenyl]-2-oxo-1, 3- oxazolidin-5-yl] methyl} acetamide [4]. Clinically used for the treatment of infections caused by multi-resistant bacteria and methicillin resistant Staphylococcus aureus (MRSA). The drug works by inhibiting the initiation of bacterial protein synthesis [2,3]. Both the drugs are marketed as combined dose tablet formulation in the ratio of 200:600 mg CEF: LNZ. Literature survey reveals that Cefixime Trihydrate can be estimated by Spectrophotometrically [5], and by HPLC[6-8] individually or with other drugs in bulk drugs and in human plasma, while Linezolid can be estimated by Spectrophotometrically [9-10], HPLC [11] in combination with other drugs. However, there is no analytical method reported for the estimation of CEF and LNZ in a combined dosage formulation. Present work describes RP-HPLC method for simultaneous estimation of CEF and LNZ in tablet formulation. The chemical structures of Cefixime trihydrate(A) and Linezolid(B) are shown in Figure 1. [1, 4]

![Figure 1](image1.png)

Figure-1: Chemical structure of (A) Cefixime Trihydrate and (B) Linezolid

MATERIALS AND METHODS

Instrumentation

Double beam UV-visible spectrophotometer (helios Alpha, Model - V 7.09) having two matched quartz cells with 1 cm light path. An Electronic analytical balance (Contech, CA34 Model) was used in the study.
Material and Reagent

Reference standard of Cefixime Trihydrate (gift sample from Sunrise Remedis Pvt Ltd, Ahmadabad, Gujarat, India) and Linezolid (gift sample from Alembic Pharmaceuticals Ltd., Gujarat, India). Tablet Zifi-turbo (F.D.C spectra healthcare Pharma, India) (Label claim: Cefixime 200 mg and Linezolid 600 mg) was used. HPLC grade methanol from Finar chemicals, Ahmadabad. All other chemicals and reagents used were of AR grade. Mili Q water was use for this study.

Preparation of Standard Stock Solution of CEF and LNZ

Accurately weighed quantity 100 mg of CEF and LNZ were transferred into separate 100 ml volumetric flask, dissolved and diluted up to mark with methanol (100 ml). This will give a stock solution having strength of 1000 μg/ml of each.

Preparation of Working Standard Solution of CEF and LNZ

100 μg/ml of CEF and LNZ solution were prepared by diluting 1 ml of stock solution to 10 ml with Methanol in separate 10 ml volumetric flask. Suitable aliquots of this solution were diluted up to the mark with methanol to get the concentration range of 2, 6, 10, 14 and 18 μg/ml for CEF and 7, 9, 11, 13 and 15 μg/ml for LNZ.

Selection of Analytical Wavelength

2-18 μg/ml solutions of CEF and 7-15 μg/ml solutions of LNZ were prepared in Methanol by appropriate dilution of working standard solution and spectrum was recorded between 200-400 nm and all zero order spectrums (D⁰) were converted to first derivative spectrum (D¹) using delta lambda 2.0 and scaling factor 20. The overlain first derivative spectrums of CEF and LNZ at different concentration were recorded. The zero crossing point (ZCP) of CEF was found to be 290 nm (Figure 2) and ZCP of LNZ was found to be 228 nm (Figure 3).

![Fig. 2: Overlain D¹ Spectrum of CEF (2-18 μg/ml) Showing Zero Crossing Point in Methanol.](image-url)
Fig. 3: Overlain D$^1$ Spectrum of LNZ (7-15 µg/ml) Showing Zero Crossing Point in Methanol.

Preparation of calibration curve

Standard solutions of CEF in the concentration range of 2 to 18 µg/ml obtained by transferring (0.2, 0.6, 1.0, 1.4 and 1.8 ml) of CEF working standard solution (100 µg/ml) to the series of 10 ml volumetric flasks and standard solutions of LNZ in the concentration range of 7 to 15 µg/ml were obtained by transferring (0.7, 0.9, 1.1, 1.3 and 1.5 ml) of LNZ working standard solution (100 µg/ml) to the series of 10 ml volumetric flasks. Then volume was adjusted up-to mark with Methanol. All dilutions were scanned in wavelength range of 200 nm to 400 nm. All zero order spectrums (D$^0$) were converted to first derivative spectrum (D$^1$). The absorbance was plotted against the respective concentrations to obtain the calibration curves.

METHODOLOGY

First order derivative method uses the Zero crossing point (ZCP) of spectra. If ZCP is more than one than we should have to consider ZCP which gives best correlation coefficient. From the overlain spectra of both drugs (as shown in figure 2 and 3), it shows that CEF and LNZ having ZCP at 290 nm and 228 nm respectively. Working standard solutions having concentration 2, 6, 10, 14, and 18 µg/ml for CEF and 7, 9, 11, 13 and 15 µg/ml for LNZ were prepared and the D$^1$ absorbance at 290 nm (ZCP of CEF) for LNZ and 228 nm (ZCP of LNZ) for CEF were measured and calibrations curve were prepared.

Calibration curve equation for,

CEF: $y = 0.0077x + 0.011$

LNZ: $y = 0.0177x + 0.081$

These both equations were used for % recovery of drug from sample solution.
RESULT AND DISCUSSION

Validation parameters

Validation of developed method was carried out as per ICH guideline.

Linearity

Appropriate volume of aliquot from CEF and LNZ working standard solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with Methanol to give solutions containing 2-18 µg/ml CEF and 7-15 µg/ml LNZ. All D\textsuperscript{1} Spectrum were recorded using above spectrophotometric condition. D\textsuperscript{1} absorbance at 290 nm and 228 nm were recorded for LNZ and CEF (Figure 4), respectively (n=6). Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Fig. 4: Overlain D\textsuperscript{1} Spectrum of CEF (2-18 µg/ml) and LNZ (7-15 µg/ml) Showing Zero Crossing Point in Methanol.

Precision

Precision of the method was determined in the terms of Repeatability, Intraday and Interday precision. Repeatability (% RSD) was assessed by analyzing test drug solution within the calibration range, six times on the same day. Intraday variation (% RSD) was determined by analysis of this solution three times on the same day. Interday precision (%RSD) was determined by analysis of this solution on three different days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by Calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

\[
LOD = 3.3 \times \frac{\sigma}{S} \quad (3)
\]
\[
LOQ = 10 \times \frac{\sigma}{S} \quad (4)
\]
Where, $\sigma$ = the standard deviation of the response and

$S$ = slope of the calibration curve.

The results of LOD and LOQ were showed in Table-5.

### Table 5: Validation Parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CEF at 228 nm</th>
<th>LNZ at 290 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability (%RSD) (n=6)</td>
<td>1.6637</td>
<td>0.4207</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day (n=3)</td>
<td>0.5710-1.7391</td>
<td>1.084-1.4547</td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>1.9441-1.9920</td>
<td>1.6589-1.9930</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.510</td>
<td>0.689</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.545</td>
<td>2.090</td>
</tr>
<tr>
<td>% Recovery (n=3)</td>
<td>98.36 % – 99.45 %</td>
<td>100.10 % – 101.66 %</td>
</tr>
<tr>
<td>Assay (mean ± S.D.) (n=5)</td>
<td>99.87 ± 0.0348</td>
<td>101.69 ± 0.1056</td>
</tr>
</tbody>
</table>

**Recovery Studies (Accuracy)**

The accuracy of the method was determined by calculating the recoveries of CEF and LNZ by the standard addition method. Known amounts of standard solutions of CEF and LNZ were at added at 80, 100 and 120 % level to pre-quantified sample solutions of CEF and LNZ and absorbance were determined at 290 nm and 228 nm set of 3 replicates. The mean % recovery was 98.36 % – 99.45 % and 100.10 % – 101.66 % for Cefixime Trihydrate and Linezolid respectively [Table-1 and 2].

### Table-1 Result of Recovery Studies for CEF

<table>
<thead>
<tr>
<th>Amount of CEF in sample (µg/ml)</th>
<th>Amount of Std CEF added (µg/ml)</th>
<th>Total amount of CEF (µg/ml)</th>
<th>Total amount of CEF found (µg/ml) Mean* ± SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>(80%) 1.6</td>
<td>3.6</td>
<td>3.54 ± 0.00020</td>
<td>98.36</td>
</tr>
<tr>
<td>2</td>
<td>(100%) 2</td>
<td>4</td>
<td>3.97 ± 0.00015</td>
<td>99.45</td>
</tr>
<tr>
<td>2</td>
<td>(120%) 2.4</td>
<td>4.6</td>
<td>4.32 ± 0.00045</td>
<td>98.38</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]
Table 2: Result of Recovery Studies for LNZ.

<table>
<thead>
<tr>
<th>Amount of LNZ in Sample (μg/ml)</th>
<th>Amount of Std LNZ Added (μg/ml)</th>
<th>Total Amount of LNZ (μg/ml)</th>
<th>Total Amount of LNZ Found (μg/ml) Mean* ± SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>(80%) 4.8</td>
<td>10.8</td>
<td>10.97 ± 0.00249</td>
<td>101.66</td>
</tr>
<tr>
<td>6</td>
<td>(100%) 6</td>
<td>12</td>
<td>12.01 ± 0.00026</td>
<td>100.10</td>
</tr>
<tr>
<td>6</td>
<td>(120%) 7.2</td>
<td>13.2</td>
<td>13.31 ± 0.00309</td>
<td>100.86</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]

Estimation of CEF and LNZ in Pharmaceutical tablet Dosage form:

Twenty tablets were weighed and powder was collected. The tablet powder equivalent to 200 mg of CEF and 600 mg of LNZ was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. This solution contains 100 μg/ml of CEF and 300 μg/ml of LNZ. The solution was filtered through Whatmann filter paper no. 41 and first few drops of filtrate were discarded. 0.2 ml of this solution was diluted to 10 ml with methanol. This solution contains 2 μg/ml of CEF and 6 μg/ml of LNZ.

The absorbance of the solution was measured at 228 nm (ZCP of LNZ) and 290 nm (ZCP of CEF) to determine concentration of CEF and LNZ respectively using first order derivative spectrophotometry and the concentration of each drug was calculated using straight line equation.

Table 3: Analysis of Marketed Formulation.

<table>
<thead>
<tr>
<th>Tablet</th>
<th>mg/tablet</th>
<th>%Recovery ± SD (% of label claim*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CEF</td>
<td>LNZ</td>
</tr>
<tr>
<td>ZIFI TURBO</td>
<td>200</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td>99.87 ± 0.0348</td>
<td>101.69 ± 0.1056</td>
</tr>
</tbody>
</table>

[*=mean value of 5 determination]

Table 4: Regression Characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CEF at 228 nm</th>
<th>LNZ at 290 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (μg/ml)</td>
<td>2-18</td>
<td>7-15</td>
</tr>
<tr>
<td>Regression Equation</td>
<td>y = 0.0077x + 0.011</td>
<td>y = 0.0177x + 0.081</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0077</td>
<td>0.0177</td>
</tr>
<tr>
<td>r²</td>
<td>0.9970</td>
<td>0.9982</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0110</td>
<td>0.0810</td>
</tr>
<tr>
<td>S.D. of Intercept</td>
<td>0.00119</td>
<td>0.00370</td>
</tr>
</tbody>
</table>
CONCLUSION

The developed method was found to be accurate, precise, simple, sensitive, and rapid and can usually be used for estimation of both these drugs in their combined dosage form. These UV methods are applicable and overcome the drawbacks of other methods which are very costly. The developed method was validated as per ICH guidelines.

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REFERENCES


