METHOD DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF CEFIXIMETRIHYDRATE AND LINEZOLID IN THEIR COMBINED TABLET DOSAGE FORM

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Abstract

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of cefixime Trihydrate (CEF) and Linezolid (LNZ) in combined tablet dosage form. The method is based on the Q analysis method and Simultaneous equations for analysis of both the drugs using methanol as solvent. First method is Q analysis method based on absorbance ratio at two selected wavelengths 278.72 nm (iso-absorptive point) and 256.70nm (λmax of linezolid). Second method is based on the simultaneous equations and wavelengths selected for analysis were 288.72 nm (λmax of Cefixime Trihydrate) and 256.70nm (λmax of Linezolid) respectively, in methanol. The linearity was obtained in the concentration range of 2-10 µg/ml and 5-25 µg/ml for both Cefixime Trihydrate and Linezolid. The results of the analysis have been validated statistically and by recovery studies. Method was found to be accurate, precise and reproducible. This method was applied to the assay of the drugs in marketed formulation, which were found in the range of 99.70% to 100.03% of the labeled value for both Cefixime Trihydrate and Linezolid in Q analysis method and 100.10% and 99.91% of the labeled value for both Cefixime Trihydrate and Linezolid in Simultaneous equation method. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.
Cefixime Trihydrate (CEF) is an oral third generation cephalosporin antibiotic. Chemically, it is \((6R,7R)-7-[(2-(2-amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetyl]amino)-3-ethenyl-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid,\) clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections. Linezolid (LNZ) is a synthetic antibacterial agent of the oxazolidinone class of antibiotics. Linezolid is chemically \(N-\{(5S)-3-[3-fluoro-4-(morpholin-4-yl) phenyl]-2-oxo-1, 3-oxazolidin-5-yl] methyl\} acetamide.\) Clinically used for the treatment of infections caused by multi-resistant bacteria including streptococcus and methicillin-resistant Staphylococcus aureus (MRSA). The drug works by inhibiting the initiation of bacterial protein synthesis. 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 \(3, HPLC4-8\) and by HPTLC9 individually or with other drugs in bulk drugs and in human plasma, while Linezolid can be estimated b spectrophotometrically \(10-11, HPLC412-13\) 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 two methods for simultaneous estimation of CEF and LNZ in tablet formulation.

**INTRODUCTION**

**MATERIALS & METHODS**

**Instrumentation**

An UV-Visible double beam spectrophotometer (SHIMADZU 1800) with 10 mm matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D), Ultrasonicator model 5.5L150H were used.

**Material used**

API of Cefixime trihydrate (gift sample from West-Coast Pharmaceutical Works LTD Ahmedabad, Gujarat, India). API of Linezolid (gift sample from Zydus Cadila Pharmaceuticals Ltd., Ankleshwar,
Gujarat, India). The pharmaceutical dosage form used in study was Zifi-turbo (label claim CEF 200 mg, LNZ 600 mg) manufactured in India by F.D.C spectra healthcare Pharma.

Reagent used
Methanol: AR grade (Finar Chemicals Pvt. Ltd, Ahmedabad, India)

Preparation of standard stock solution
Accurately weighed 10mg of quantity of Cefixime Trihydrate & Linezolid was transferred into 10 ml volumetric flask and dissolved in methanol and diluted up to the mark with methanol to give a stock solution having strength 1mg/ml (1000μg/ml). 100 μg/ml of Cefixime Trihydrate & Linezolid working standard solution was prepared by diluting 1 ml of stock solution with methanol in 10 ml volumetric flask up to the mark.

METHODS
(1)Q ABSORBANCE RATIO
It uses the ratio of absorbances at two selected wavelengths, one which is anis absorptive point and other being the λ-max of one of the two components. From the CEF and LNZ show an isoabsorptive point at 278.72 nm. The second wavelength used is 256.70 nm, which is the λ-max of LNZ. Six working standard solutions having concentration 2,4,6,8,10μg/ml for CEF and 5,10,15,20,2 μg/ml for LNZ were prepared in methanol and the absorbances at 278.72 nm (isoabsorptive point) and 256.70 nm (λ-max of LNZ) were measured and absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance/ Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

\[
\begin{align*}
CX &= \left(\frac{QM - Qy}{QX - QY}\right) \times A1/ax1........... (1) \\
CY &= \left(\frac{A1/ax1}{CX}\right) - CX ............... (2)
\end{align*}
\]

Where, A1 and A2 are absorbances of mixture at 278.72 nm and 256.70 nm; aX1 and aY1 are absorptivities of CEF and LNZ at 278.72 nm; aX2 and aY2 are absorptivities of CEF and LNZ respectively at 256.70 nm;

\[
QM = A2 / A1,
\]
\[
QA = aX2 / aX1
\]
QI = aY2 / aY1.

(2) SIMULTANEOUS EQUATION METHOD:

For the simultaneous equation method wavelengths selected were λmax of both the drugs, at the λmax of the LNZ, CEF shows the considerable absorbance and at the λmax of CEF, LNZ shows considerable absorbance. The study of spectra also reveals that CEF and LNZ have λmax at 288.72 nm and at 256.70 nm respectively. Both the drugs were found to have considerable absorbance at λmax of each other. The wavelengths selected for analysis were 288.72 nm and 256.70 nm respectively for CEF and LNZ. A series of standard solutions ranging from 2-10μg/ml for CEF and from 5-25μg/ml for LNZ both were prepared and the absorbance of solutions was recorded at selected wavelengths. Calibration curve of absorbance versus concentration was plotted. The Calibration curves were found to be linear in the concentration range under study. The concentration of two drugs in mixture was calculated by using following equations:

\[
CX = \frac{(A2ay1 – A1ay2)}{(ax2ay1 – ax1ay2)}... (3)
\]

\[
CY = \frac{(A1ax2 – A2ax1)}{(ax2ay1 – ax1ay2)}... (4)
\]

Where A1 and A2 are the absorbances of mixture at 288.72 nm and 256.70 nm and ax1, ay1, ax2 and ay2 were absorptivity of CEF and LNZ at 288.72 nm and 256.70 nm respectively.

VALIDATION OF THE Q ABSORPTION RATIO AND SIMULTANEOUS EQUATION METHOD:

These methods were validated with respect to linearity, accuracy, intraday and interday precision, limit of detection (LOD) and limit of quantitation (LOQ), in accordance with ICH guideline.

Linearity

Linearity was taken for Cefixime Trihydrate and Linezolid in the concentration range of 2-10 μg/ml & 5-25 μg/ml respectively to both methods. The calibration curve was obtained by plotting absorbance → concentrations.

Precision

For Intraday precision, it was carried out by preparing 3 replicates of 4, 6 and 8μg/ml & 10, 15 & 20 μg/ml of CEF and LNZ
concentrations, within the linearity range in both method and measuring the absorbance of each solution. % RSD (% relative standard deviation) was calculated. For Interday precision, 3 different concentration solutions within the linearity range were measured for 3 different days in both methods. % RSD (% relative standard deviation) was calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)
They were calculated as 3.3 $\sigma$/S and 10 $\sigma$/S respectively. Where $\sigma$ is the standard deviation of the response (y- intercept) and S, is the mean of the slope of calibration plot.

Accuracy
To study accuracy of the method, recovery studies were carried out by addition of standard drug in a tablet sample at 0%, 50%, 100% and 150%. The percentage of recovery was calculated in both methods.

Assay
It was tested by analysis of commercially available marketed formulation. Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 200mg of Cefixime Trihydrate was transferred to 50ml volumetric flask containing 40 ml of methanol, gentle shaking was carried out for 5 min and ultrasonicated for 5 min. The volume was made up to the mark with methanol. The tablet sample solution was filtered through Whatman filter paper no. 41. 5 ml of filtrate was further diluted to 25 ml of methanol to get 100 $\mu$g/ml. From the Concentrate 100 $\mu$g/ml of sample stock solution take 0.6 ml of solution of Cefixime Trihydrate and 1.5ml of Linezolid diluted up to the mark in 10 ml volumetric flask. So the final solution was made which contains 6 $\mu$g/ml Cefixime Trihydrate and15$\mu$g/ml Linezolid both. absorbances were measured at 288.78 nm, 256.70 nm and 278.72 nm against blank. The concentrations of two drugs in sample were determined by using equations 1 and 2 Q-absorbance analysis and equation 3 and 4 in simultaneous equation method.

RESULTS AND DISCUSSION

Method I: Simultaneous Equation Method
UV-spectrophotometric method using simultaneous equation was developed. CEF showed absorbance maxima at 288.72 nm and LNZ at 256.70 nm. Linearity was observed in the concentration range of 2-10 μg/ml for CEF and 5-25 μg/ml for LNZ. Correlation coefficient was found to be 0.997 and 0.996 at 288.72 nm and 256.70 nm respectively. The proposed method was applied for pharmaceutical formulation and % label claim for CEF and LNZ was found to be 100.10 and 99.91 respectively. The method is accurate and precise and can be used for routine pharmaceutical analysis.

Method II: Absorbance Ratio Method

UV-spectrophotometric method by using absorbance ratio method was developed. Absorbances selected were 278.72 nm (isoabsorptive point) and 256 nm (λ max of Linezolid). Linearity was observed in the concentration range of 2-10 μg/ml and 5-25 μg/ml. Correlation coefficient was found to be 0.998 and 0.996 respectively. The proposed method was applied for pharmaceutical formulation; % label claim for CEF and LNZ was found to be 99.70 and 100.03 respectively.

CONCLUSION

The proposed Q absorption method and simultaneous equation method provides simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of CEF and LNZ in combined tablet dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The method can be used for routine analysis of CEF and LNZ in combined dosage form.

ACKNOWLEDGEMENT

The authors are thankful to West coast pharmaceutical ltd & zydus cadila healthcare ltd. to give a gift sample CEF and LNZ respectively to carry out research work. The authors are highly thankful to K. B. Raval College of pharmacy, Shertha, Kasturinagar for provide all facilities to carry out research work.
Figure 1 Chemical structure of cefixime trihydrate

Figure 2 Chemical structure of Linezolid
Figure 3 Overlay spectra of CEF at 288.72nm

Figure 4 Overlay spectra of LNZ at 256.70nm
Figure 5 Overlay spectra of CEF (6 μg/ml) and LNZ (15 μg/ml)

Table 1.1 Summary of Validation Parameters of Q- Absorbance ratio

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CEF</th>
<th>LNZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>278.72nm</td>
<td>256.70nm</td>
</tr>
<tr>
<td>Recovery %</td>
<td>99.72-100.44</td>
<td>98.95-100.22</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
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<tr>
<td>Intra-day (n=3)</td>
<td>0.13-0.26</td>
<td>0.11-0.21</td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>0.13-0.27</td>
<td>0.11-0.21</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>0.2</td>
<td>0.11-0.21</td>
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<tr>
<td>LOQ (μg/ml)</td>
<td>0.6</td>
<td>3</td>
</tr>
<tr>
<td>Solvent suitability</td>
<td>24hrs</td>
<td>24hrs</td>
</tr>
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</table>
Table 1.2 Statistical Data CEF and LNZ by Q- Absorbance ratio

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CEF</th>
<th></th>
<th>LNZ</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Wavelength</td>
<td>278.72</td>
<td>256.70</td>
<td>278.72</td>
<td>256.70</td>
</tr>
<tr>
<td>Range</td>
<td>(2-10μg/ml)</td>
<td>(2-10μg/ml)</td>
<td>(5-25μg/ml)</td>
<td>(5-25μg/ml)</td>
</tr>
<tr>
<td>Slope</td>
<td>0.031</td>
<td>0.025</td>
<td>0.012</td>
<td>0.048</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.013</td>
<td>0.021</td>
<td>0.12</td>
<td>0.049</td>
</tr>
<tr>
<td>Regression Coefficient</td>
<td>0.998</td>
<td>0.991</td>
<td>0.996</td>
<td>0.996</td>
</tr>
</tbody>
</table>

Table 1.3 Accuracy Data for CEF and LNZ by Q- Absorbance Method

<table>
<thead>
<tr>
<th>% Level</th>
<th>Amount of Drug taken</th>
<th>Amount of Drug Added</th>
<th>Amount Recovered</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3</td>
<td>9</td>
<td>1.5</td>
<td>4.5</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>150</td>
<td>3</td>
<td>9</td>
<td>4.5</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Table 1.4 Assay Results of Marketed Formulation

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Drug</th>
<th>Labeled claim (mg)</th>
<th>Amount found (mg)</th>
<th>% label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zifi-turbo</td>
<td>Cefixime trihydrate</td>
<td>200</td>
<td>199.40</td>
<td>99.70</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>600</td>
<td>600.20</td>
<td>100.03</td>
</tr>
</tbody>
</table>
Table 2.1 Summary of Validation Parameters of simultaneous equation method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CEF</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CEF LNZ</td>
<td>288.72</td>
<td>256.70</td>
</tr>
<tr>
<td>Recovery %</td>
<td>98.95-100.22</td>
<td>98.05-100.22</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day (n=3)</td>
<td>0.146-0.296</td>
<td>0.11-0.21</td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>0.118-0.224</td>
<td>0.118-0.214</td>
</tr>
<tr>
<td>LOD(µg/ml)</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.14</td>
<td>3</td>
</tr>
<tr>
<td>Solvent suitability</td>
<td>24hrs</td>
<td>24hrs</td>
</tr>
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Table 2.2 Statistical Data CEF and LNZ by simultaneous equation Method:

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<td>Analytical Wavelength</td>
<td>288.72</td>
<td>256.70</td>
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<tr>
<td>Range</td>
<td>2-10µg/ml</td>
<td>2-10µg/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.034</td>
<td>0.025</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.013</td>
<td>0.021</td>
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<tr>
<td></td>
<td>CEF(μg/ml)</td>
<td>LNZ(μg/ml)</td>
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</tr>
<tr>
<td>50</td>
<td>3</td>
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<td>100</td>
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<td>200</td>
<td>200.20</td>
<td>100.10</td>
</tr>
<tr>
<td></td>
<td>Linezolid</td>
<td>600</td>
<td>599.50</td>
<td>99.91</td>
</tr>
</tbody>
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