SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND LINEZOLID IN 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 and Linezolid in bulk and tablet dosage form. The method is based on the simultaneous equations for analysis of both the drugs using 0.05 M Potassium phosphate buffer pH 7.2 as solvent. Cefixime Trihydrate has absorbance maxima at 287.20 nm and Linezolid has absorbance maxima at 250.60 nm in the pH 7.2. The linearity was obtained in the concentration range of 2-22 μg/ml and 2-18 μg/ml for Cefixime Trihydrate and Linezolid, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 100.2 ± 0.56 and 101.23 ± 0.63 for Cefixime Trihydrate and Linezolid, respectively. The method was successfully applied to tablet dosage form. The suitability of this method for the quantitative determination of Cefixime Trihydrate and Linezolid was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of Cefixime Trihydrate and Linezolid in combination. The results of analysis have been validated statistically and by recovery studies.

KEY WORDS: Cefixime Trihydrate, Linezolid, Recovery, Simultaneous equations method, Validation.

INTRODUCTION
Cefixime (CEF) is an oral third generation cephalosporin class of antibiotic. Chemically, it is (6R, 7R)-7-[[2-(2-amino-1,3-thiazol-4-yl)-2(carboxymethoxy-imino)acetyl]amino]-3-ethyl-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. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States Pharmacopoeia (USP), European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP). Literature survey reveals that Cefixime can be estimated by spectrophotometrically, TLC, HPTLC, HPLC and HPCE individually and in combination with other drugs in bulk drugs, in human plasma, and Linezolid (LIN) is first of the oxazolidinone class of antibiotic drug and chemically it is N-[(5S)-3-[3-fluoro-4(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-yl][methyl]acetamide and it is also useful as Antibacterial Agents. (Figure 2). Linezolid (LIN) is official in Indian Pharmacopoeia (IP). Literature survey reveals Linezolid can be estimated by spectrophotometrically, HPLC and HPTLC individually and in combination with other drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CEF and LIN in their combined dosage forms. Literature survey reveals some simple spectrophotometric method for simultaneous estimation of CEF and LIN in dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on simultaneous equations for simultaneous estimation of both drugs in their combined tablet dosage form.

MATERIALS AND METHODS

Apparatus
A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials
CEF and LIN bulk powder was kindly gifted by Welable Healthcare, Mehsana, Gujarat, India. Potassium Phosphate Monobasic (Extra pure, Finar Chemicals Ltd. Ahmedabad), Sodium Hydroxide(SD. Fine Chem Ltd., Mumbai) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of solvent
An accurately weighed 6.8 gm of potassium phosphate monobasic was dissolved in 1000 ml distilled water and pH 7.2 was adjusted with 1 N Sodium Hydroxide solution.

Preparation of 1N Sodium Hydroxide Solution
An accurately weighed 40 gm Sodium Hydroxide Pallets were dissolved in 1000 ml distilled water.

Preparation of standard stock solutions
An accurately weighed quantity of standard CEF (10 mg) and LIN (10 mg) powder were transferred to 100 ml separate volumetric flasks and dissolved in 0.05 M Potassium phosphate buffer pH 7.2. The flasks were sonicated for 5 minutes and volumes were made up to mark with 0.05 M Potassium phosphate buffer pH 7.2 to give a solution containing 100 μg/ml each of CEF and LIN.

Methodology
The working standard solutions of CEF and LIN were prepared separately in 0.05 M Potassium phosphate buffer pH 7.2 having concentration of 10 μg/ml. They were scanned in the wavelength range of 200-400 nm against 0.05 M Potassium phosphate buffer pH 7.2 as blank. Maximum absorbance was obtained at 287.20 nm and 250.60 nm for CEF and LIN, respectively. These two wavelengths can be employed for the determination of CEF and LIN without any interference from the other components in their marketed formulations.

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Validation of the proposed method
The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines\(^1\)

**Linearity (calibration curve)**

The calibration curves were plotted over a concentration range of 2.22 \(\mu g/ml\) for CEF and 2.18 \(\mu g/ml\) for LIN. Accurately measured standard solutions of CEF (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 ml) and LIN (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with 0.05 M Potassium phosphate buffer pH 7.2. The absorances of the solutions were measured at 287.20 and 250.60 nm against 0.05 M Potassium phosphate buffer pH 7.2 as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

**Method precision (repeatability)**

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions \((n = 6)\) for CEF and LIN (12 \(\mu g/ml\) for both drugs) without changing the parameter of the proposed spectrophotometry method.

**Intermediate precision (reproducibility)**

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CEF and LIN (8, 12, 16 \(\mu g/ml\) for CEF and 8, 12, 16 \(\mu g/ml\) for LIN). The result was reported in terms of relative standard deviation \((%\ RSD)\).

**Accuracy (recovery study)**

The accuracy of the method was determined by calculating recovery of CEF and LIN by the standard addition method. Known amounts of standard solutions of CEF and LIN were added at 50, 100 and 150% level to prequantified sample solutions of CEF and LIN (5 \(\mu g/ml\) CEF and 15 \(\mu g/ml\) LIN). The amounts of CEF and LIN were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

**Limit of detection and Limit of quantification**

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\(^1\)

\[
\text{LOD} = 3.3 \times \sigma/S \\
\text{LOQ} = 10 \times \sigma/S
\]

Where, \(\sigma\) = the standard deviation of the response and \(S\) = slope of the calibration curve

**Analysis of CEF and LIN from Tablet dosage form**

Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 50 mg of CEF was transferred to 100.0 ml volumetric flask, 0.05 M Potassium phosphate buffer pH 7.2 added, sonicated for 20 minutes and volume was made-up to the mark with 0.05 M Potassium phosphate buffer pH 7.2. The solution was then filtered through a Whatman filter paper (No.41). The filtrate was further diluted with 0.05 M Potassium phosphate buffer pH 7.2 to obtain 5 \(\mu g/ml\) of CEF and 15 \(\mu g/ml\) of LIN. The concentration of both CEF and LIN were determined by measuring the absorbance of the sample at 287.20nm, 250.60nm. Concentration of sample solution was determined by Simultaneous equation method.

\[
C_x = (A_2 a Y_1 - A_1 a Y_2) / (a Y_1 a X_2 - a Y_2 a X_1) \\
C_y = (A_1 a X_2 - A_2 a X_1) / (a Y_1 a X_2 - a Y_2 a X_1)
\]

Where, \(A_1\) and \(A_2\) are absorbances of mixture at 250.60 nm and 287.20 nm; \(a X_1\) and \(a Y_1\) are absorptivities of LIN and CEF respectively at 250.60 nm; \(a X_2\) and \(a Y_2\) are absorptivities of LIN and CEF respectively at 287.20 nm.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CEF</th>
<th>LIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>287.20</td>
<td>250.60</td>
</tr>
<tr>
<td>Beer’s law limit ((\mu g/ml))</td>
<td>2.22</td>
<td>2.22</td>
</tr>
<tr>
<td>Regression equation ((y = a + bc))</td>
<td>(y = 0.0515x + 0.0138)</td>
<td>(y = 0.0321x + 0.0154)</td>
</tr>
<tr>
<td>Slope ((b))</td>
<td>0.0515</td>
<td>0.0321</td>
</tr>
<tr>
<td>Intercept ((a))</td>
<td>0.0138</td>
<td>0.0154</td>
</tr>
<tr>
<td>Correlation coefficient ((R^2))</td>
<td>0.9974</td>
<td>0.996</td>
</tr>
<tr>
<td>LOD ((\mu g/ml))</td>
<td>0.34</td>
<td>0.38</td>
</tr>
<tr>
<td>LOQ ((\mu g/ml))</td>
<td>1.02</td>
<td>1.14</td>
</tr>
<tr>
<td>Repeatability ((%\ RSD, n = 6))</td>
<td>0.73</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Intraday</td>
<td>Interday</td>
</tr>
<tr>
<td></td>
<td>0.47-0.91%</td>
<td>0.46-0.86%</td>
</tr>
<tr>
<td></td>
<td>0.58-0.95%</td>
<td>0.53-1.07%</td>
</tr>
<tr>
<td>Accuracy (\pm\ S.\ D.) ((%\ Recovery, n = 5))</td>
<td>99.91 ± 1.25</td>
<td>99.48 ± 0.57</td>
</tr>
</tbody>
</table>

**Table 2**: Recovery data of CEF and LIN

For CEF: 50 \(\mu g/ml\) sample solution, 100 \(\mu g/ml\) standard stock solution
For LIN: 50 \(\mu g/ml\) sample solution, 100 \(\mu g/ml\) standard stock solution

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken ((\mu g/ml))</th>
<th>Amount added (%)</th>
<th>% Recovery (\pm\ S.\ D.) ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEF</td>
<td>5</td>
<td>50</td>
<td>100.8 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100</td>
<td>99.4 ± 0.61</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>150</td>
<td>100.4 ± 0.73</td>
</tr>
<tr>
<td>LIN</td>
<td>5</td>
<td>50</td>
<td>100.8 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100</td>
<td>102.0 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>150</td>
<td>100.9 ± 0.62</td>
</tr>
</tbody>
</table>

S. D. = Standard deviation. \(n\) = Number of determinations.
RESULTS

The standard solutions of CEF and LIN were scanned separately in the UV range and zero-order spectra for CEF and LIN were recorded. Maximum absorbance was obtained at 287.20 nm and 250.60 nm for CEF and LIN, respectively. These two wavelengths can be employed for the determination of CEF and LIN without any interference from the other drug in their combined synthetic mixture. Overlain zero-order absorption spectrum of CEF and LIN in methanol is shown in (Figure 3). Linear correlation was obtained between absorbances and concentrations of CEF and LIN in the concentration ranges of 2-22 µg/ml and 2-18 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of 0.99 ± 0.31 for CEF and 0.97 ± 0.31 for LIN in methanol. The RSD values of CEF and LIN were found to be 0.98 and 0.97 % at 287.20 and 250.60 nm, respectively. The RSD value of LIN was found to be 1.44 and 0.77 % at 287.20 and 250.60 nm, respectively. Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable. The low RSD values of interday (0.47-0.91%) and 0.46-0.86% for CEF at 287.20 and 250.60 nm, respectively and 0.77-0.99% and 0.49-0.71% for LIN at 287.20 and 250.60 nm, respectively) and intraday (0.58-0.95% and 0.53-1.07% for CEF at 287.20 and 250.60 nm, respectively and 0.86-1.49% and 0.51-0.82% for LIN at 287.20 and 250.60 nm, respectively) variation for CEF and LIN, reveal that the proposed method is precise. LOD and LOQ values for CEF were found to be 0.34 and 1.02 µg/ml and 0.38 and 1.14 µg/ml at 287.20 and 250.60 nm, respectively. LOD and LOQ values for LIN were found to be 0.43 and 1.31 µg/ml and 0.21 and 0.64 µg/ml at 287.20 and 250.60 nm, respectively. These data show that the method is sensitive for the determination of CEF and LIN. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.2 ± 0.58 and 101.23 ± 0.64 for CEF and LIN, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine CEF and LIN in their combined tablet dosage form. The results obtained for CEF and LIN were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of CEF and LIN in synthetic mixture as well as in pharmaceutical dosage forms.

DISCUSSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of CEF and LIN in Synthetic mixture. The method utilizes easily available and cheap solvent for analysis of CEF and LIN hence the method was also economic for estimation of CEF and LIN from Synthetic mixture. The common
excipients and other additives in the dosage form do not interfere in the analysis of CEF and LIN in method; hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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REFERENCES
3. Indian pharmacopoeia, Government of India, the Controller Publication, New Delhi, Addendum 2008; 2377-78.

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