Simultaneous Estimation of Cefuroxime Axetil and Linezolid by Three Novel Spectrophotometric Methods in Pharmaceutical Dosage Form and their Comparison Using ANOVA

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ABSTRACT
Three simple, accurate, sensitive, precise and economical UV spectrophotometric methods (A, B & C) have been developed for simultaneous estimation of cefuroxime axetil and linezolid in pharmaceutical dosage form and their comparision using ANOVA. Method A employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 277 nm and 257 nm which are the λmax values of cefuroxime axetil and linezolid, respectively in methanol. Method B is based on the principle of Q-absorbance ratio where in the absorbance was measured at 272 nm (iso-absorptive point) and 257 nm (λmax of linezolid) in methanol. The linearity was obtained in the concentration ranges of 3-11 and 3.6 – 13.2 μg/ml, respectively. Method C is based on the first derivative spectrophotometric method at zero crossing wavelengths. In this method the zero crossing point of cefuroxime axetil was selected at 256 nm and for linezolid it was 275 nm. The linearity was obtained in the concentration range of 10-30 μg/ml for cefuroxime axetil and 12-36 μg/ml for linezolid. The accuracy and precision of the methods were determined and validated statistically. All the methods showed good reproducibility and recovery with % RSD less than 2. The three methods were compared using one -way ANOVA and the f values of linezolid was found to be less than ftab value indicating that there is no significant difference in the assay results by the three methods. All methods were found to be rapid, specific, precise and accurate and these methods require no preliminary separation and found no interferences from the tablet excipients so it can be used for routine analysis of both drugs in quality control laboratories.

Keywords: Cefuroxime axetil, Linezolid, Simultaneous equation, Q-absorbance ratio, First derivative spectrophotometric, validation.

INTRODUCTION
Cefuroxime axetil (CEF) is a second generation oral cephalosporin antibiotic used for acute otitis media, bone and joint infections, meningitis, pharyngitis and tonsillitis, respiratory tract infections, septicemia, skin and skin structure infections1,2. It is chemically (6R, 7R) – 3 – carboxamidoxymethyl 1 – 7 - [Z]-2-(2-furyl) – 2 - (methoxyimino) acetamido – ceph – 3 - em-4-carboxylic acid3 (Figure 1). CEF is official in Indian Pharmacopoeia (IP)4, British Pharmacopoeia (BP)5 and United State Pharmacopoeia (USP)6. IP, BP and USP describes liquid chromatography for the estimation of CEF. Linezolid (LZD) is an oxazolidinone antibiotic (anti-bacterial) which inhibits bacterial protein synthesis7. It is chemically N{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methylacetamide} (Figure 2)7. The deep literature survey reveals that various spectrophotometric and chromatographic methods8-22 available for the estimation of CEF and LZD alone and in combination with other drugs and in biological fluids. Combination of cefuroxime axetil and linezolid is not official in any pharmacopoeias and hence no official method available for analysis of both drugs in combination. Literature survey also reveals that there are no reported methods available for simultaneous estimation of CEF and LZD in combined dosage form. Therefore, simple, rapid, and reliable method for simultaneous estimation of these drugs in nasal drops seemed to be necessary. Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. The purpose of this study was to determine both drugs concurrently by simple, accurate, rapid and precise simultaneous equation, Q-absorbance ratio and first derivative spectrophotometric assays for routine analysis.

MATERIALS AND METHODS
Apparatus and instrument
UV visible double beam spectrophotometer (SHIMADZU -1800, Japan) with software UV Probe 2.33, with spectral slit width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1cm matched quartz cells

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and digital balance Shimadzu ATX 224, Japan and ultrasonicator were used. Volumetric flasks and pipettes of borosilicate glasses were used in the study.  

**Table 1: Assay results for tablets using the proposed methods**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Proposed methods</th>
<th>Label Claim (mg)</th>
<th>Amount of drug found (mg)</th>
<th>% Label Claim Assay (n=3) ± SD (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>METHOD A</td>
<td>500 600</td>
<td>496.33 594.66</td>
<td>98.66±0.55 99.11±0.25</td>
</tr>
<tr>
<td></td>
<td>METHOD B</td>
<td>500 600</td>
<td>495.33 597.83</td>
<td>99.06±0.27 99.63±0.19</td>
</tr>
<tr>
<td></td>
<td>METHOD C</td>
<td>500 600</td>
<td>493.33 596.33</td>
<td>99.26±0.48 99.38±0.40</td>
</tr>
</tbody>
</table>

**Table 2: Application of the standard addition technique to the analysis of CEF and LZD in tablets by the proposed methods**

<table>
<thead>
<tr>
<th>Proposed methods</th>
<th>Concentration of drug taken (μg/ml)</th>
<th>Concentration of drug added (μg/ml)</th>
<th>Concentration of drug found (μg/ml)</th>
<th>% Recovery (n=3) ± SD (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METHOD A</td>
<td>5 6 2.5 3</td>
<td>7.5 9</td>
<td>99.46±0.23 99.44±1.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 6 5 6</td>
<td>10 12</td>
<td>99.53±0.11 99.44±1.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 6 7.5 9</td>
<td>12.5 15</td>
<td>99.53±0.11 100.55±1.91</td>
<td></td>
</tr>
<tr>
<td>METHOD B</td>
<td>5 6 2.5 3</td>
<td>7.5 9</td>
<td>99.26±0.11 100.50±1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 6 5 6</td>
<td>10 12</td>
<td>99.40±0.20 99.44±1.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 6 7.5 9</td>
<td>12.5 15</td>
<td>99.50±0.21 100.55±0.81</td>
<td></td>
</tr>
<tr>
<td>METHOD C</td>
<td>15 18 7.5 9</td>
<td>22.5 27</td>
<td>99.86±0.90 99.63±0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 18 15 18</td>
<td>30 36</td>
<td>99.66±0.35 100.36±0.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 18 22.5 27</td>
<td>37.5 45</td>
<td>99.70±0.80 100.43±0.57</td>
<td></td>
</tr>
</tbody>
</table>

**Chemicals and reagents**

Pure drugs samples of cefuroxime axetil and linezolid were kindly supplied as a gift sample from Bharat.
Pareneterals ltd, Vadodara and Alembic Pharmaceuticals ltd, Vadodara, respectively. Methanol (AR Grade) and other reagent were provided by Department of Quality Assurance, Pioneer Pharmacy Degree College, Vadodara, Gujarat, India. Marketed formulation (tablets containing CEF 500 mg and LZD 600 mg) was purchase from local market.

Selection of common solvent

Methanol of analytical reagent grade was selected as a common solvent for developing spectral characteristics of both drugs. The selection was made after assessing the solubility of both drugs in different solvents like water, chloroform, ether etc.

Preparation of standard stock solutions of CEF and LZD

Accurately weighed quantities of CEF (10 mg) and LZD (10 mg) transferred to separate volumetric flasks (100 ml), dissolved in methanol (small quantity) and diluted up to mark with methanol (100 μg/ml of CEF and LZD).

Methodology

Method A: Simultaneous Equation Method

In quantitative estimation of two components by Simultaneous Equation method, two wavelengths i.e.277 nm of CEF and 257nm of LZD were selected as their respective λmax from the overlap spectrum (Figure 3-6) at which both drugs have absorbance. For simultaneous equation method, working standard solutions having concentrations 3, 5, 7, 9 and 11 μg/ml for CEF and 3.6, 6, 8.4, 10.8 and 13.2 μg/ml for LZD were prepared in methanol using the stock solutions. A set of two simultaneous equations were formed using absorptivity coefficients at selected wavelengths. The concentrations of two drugs in the mixture were calculated using the following two simultaneous equations. Statistical parameters like the slope, intercept coefficient correlation, standard deviation and relative standard deviation were calculated.

\[
C_1 = A_1 y_1 / a_1 y_2 ax_1
\]

\[
C_2 = Q_m
\]

For simultaneous equations were formed using absorptivity coefficients at selected wavelengths. The concentrations of two drugs in the mixture were calculated using the following two simultaneous equations. Statistical parameters like the slope, intercept coefficient correlation, standard deviation and relative standard deviation were calculated.

\[
C_1 = A_1 y_1 / a_1 y_2 ax_1
\]

\[
C_2 = Q_m
\]

In the quantitative assay of two components by Q absorbance ratio method, absorbances were measured at the selected wavelength, i.e., 272 nm (isobestic point) and 257 nm (wavelength of maximum absorption of LZD) (Figure 3). Working standard solutions having concentration 3, 5, 7, 9 and 11 μg/ml for CEF and 3.6, 6, 8.4, 10.8 and 13.2 μg/ml for LZD were prepared in methanol using stock solutions and the absorbances at 272 nm (isoabsorptive point) and 257 nm, (λ-max of LZD) were measured and the concentration of each component can be calculated by mathematical treatment of the following mentioned equation.

\[
Cy = A_1 ax 2 / ay 1 ax 2
\]

\[
C_1 = Q_m - Qy/Qx - Qy. A_1/a_2\text{…(3)}
\]

For LZD,

\[
C_2 = Q_m - Qx/Qy - Qx. A_1/a_2\text{…(4)}
\]

where, C 1 = concentration of CEF

C 2 = concentration of LZD

### Table 3: Summary of validation parameter by developed method

<table>
<thead>
<tr>
<th>Methods</th>
<th>Drug</th>
<th>Parameters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Repeatability (RSD, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METHOD A</td>
<td>CEF (277 nm)</td>
<td>Slope</td>
<td>0.0374</td>
<td>Intercep</td>
<td>0.0116</td>
<td>Correlation coefficient</td>
<td>0.9998</td>
<td>LOD (μg/ml)</td>
</tr>
<tr>
<td></td>
<td>CEF (257nm)</td>
<td></td>
<td>0.0273</td>
<td>0.0067</td>
<td>0.9994</td>
<td>0.13</td>
<td>3.0</td>
<td>0.22 -1.07</td>
</tr>
<tr>
<td></td>
<td>LZD (277nm)</td>
<td></td>
<td>0.0199</td>
<td>0.0067</td>
<td>0.9971</td>
<td>0.19</td>
<td>3.6</td>
<td>0.67 -1.24</td>
</tr>
<tr>
<td></td>
<td>LZD (257 nm)</td>
<td></td>
<td>0.0648</td>
<td>0.0103</td>
<td>0.9993</td>
<td>0.16</td>
<td>3.6</td>
<td>0.21 -0.67</td>
</tr>
<tr>
<td></td>
<td>CEF (272 nm)</td>
<td></td>
<td>0.0366</td>
<td>0.0109</td>
<td>0.9995</td>
<td>0.12</td>
<td>3.0</td>
<td>0.21 -1.08</td>
</tr>
<tr>
<td>METHOD B</td>
<td>CEF (257 nm)</td>
<td></td>
<td>0.0273</td>
<td>0.0067</td>
<td>0.9994</td>
<td>0.10</td>
<td>3.0</td>
<td>0.45 -0.80</td>
</tr>
<tr>
<td></td>
<td>LZD (272 nm)</td>
<td></td>
<td>0.0312</td>
<td>0.0079</td>
<td>0.9998</td>
<td>0.16</td>
<td>3.6</td>
<td>0.30 -0.43</td>
</tr>
<tr>
<td></td>
<td>LZD (257 nm)</td>
<td></td>
<td>0.0648</td>
<td>0.01030</td>
<td>0.9993</td>
<td>0.10</td>
<td>3.6</td>
<td>0.17 -0.39</td>
</tr>
<tr>
<td>METHOD C</td>
<td>CEF (275 nm)</td>
<td></td>
<td>0.0015</td>
<td>0.0108</td>
<td>0.9995</td>
<td>0.14</td>
<td>10</td>
<td>0.23 -0.47</td>
</tr>
<tr>
<td></td>
<td>LZD (256 nm)</td>
<td></td>
<td>0.004</td>
<td>0.0306</td>
<td>0.9997</td>
<td>0.15</td>
<td>12</td>
<td>0.06 -0.11</td>
</tr>
</tbody>
</table>

In quantitative estimation of two components by Q-absorbance ratio method, absorbances were measured at two wavelengths, one being the isobestic wavelength and the other being wavelength of maximum absorption of one of the two components. From overlap spectra of CEF and LZD, absorbances were measured at the selected wavelength, i.e., 272 nm (isobestic point) and 257 nm (wavelength of maximum absorption of LZD) (Figure 3). Working standard solutions having concentration 3, 5, 7, 9 and 11 μg/ml for CEF and 3.6, 6, 8.4, 10.8 and 13.2 μg/ml for LZD were prepared in methanol using stock solutions and the absorbances at 272 nm (isoabsorptive point) and 257 nm, (λ-max of LZD) were measured and the concentration of each component can be calculated by mathematical treatment of the following mentioned equation.
A 1 = absorbance of sample at isoabsorptive wavelength (272 nm)
a = absorptivity of CEF and LZD at isoabsorptive wavelength (272 nm)
Qx = absorptivity of CEF at 257 nm/absorptivity of CEF at 272 nm
Qy = absorptivity of LZD at 257 nm/absorptivity of LZD at 272 nm
Qm = absorptivity of sample solution at 257 nm/absorptivity of sample solution at 272 nm.

Method C: First Derivative Spectroscopy Method
For first derivative spectrophotometric method, accurate aliquots of CEF equivalent to 10-30 µg/ml were transferred from its stock solution (100 µg/ml) into a series of 10 ml volumetric flasks and diluted to mark with methanol and mixed well. Accurate aliquots of LZD equivalent to 12-36 µg/ml were transferred from its working solution (100 µg/ml) into a series of 10 ml volumetric flasks and diluted to mark with methanol and mixed well. Considering all the derivative order spectra of CEF and LZD from first to fourth derivative, the first derivative order spectra with d (N) =2 was found suitable. The zero crossing point on the first derivative spectra of one drug, the other drug shows substantial absorbance, these two wavelengths can be employed for the estimation of CEF and LZD without any interference from other drug in combined formulations. From the derivatised spectra of prepared mixtures the absorbances were measured at 256 nm for CEF and 275 nm for LZD. These absorbances Vs concentration were plotted in the quantitative mode to obtain the working curves from which by extrapolating the value of absorbances of the sample solution, the concentration of the corresponding drugs were determined. Both the drugs obeyed Beer's Law.

Analysis of CEF and LZD in tablets
Marketed tablets formulations containing CEF (500 mg) and LZD (600 mg) were analyzed using these three methods. From the triturate of 20 tablets, an equivalent to 10 mg of CEF and 12 mg of LZD was weighed and dissolved in 10 ml of methanol in 100 ml volumetric flask by sonication for 15 mins. Then final volume of the solution was made up to 100 ml with methanol to get final concentration of 100 µg/mL of CEF and 120 µg/mL of LZD. The solution was filtered through whatmann filter paper no.41 and filtrate was appropriately diluted to get approximate concentration of 5 µg/mL of CEF and 6 µg/mL of LZD for method A & B and 10 µg/mL of CEF and 12 µg/mL of LZD for method C. Absorbances of sample solutions were recorded at 277 nm and 257 nm.
and the concentration of two drugs in the sample were determined by using eqns.1 and 2 (Method-A). Absorbances of sample solutions were recorded at 272 nm (Isoabsorptive Point) and 257 nm (λ max of LZD) and concentration of two drugs in the sample were determined by using equations 3 and 4 (Method B). For First derivative method (Method C) the absorbance was measured at 256 nm (ZCP of LZD) and 275 nm (ZCP of CEF). The concentration of each analyte was determined with the equations generated from calibration curve of respective drugs.

Validation parameters
Validation was carried out according to ICH guideline.

Accuracy
For studying the accuracy of the proposed methods, and for checking the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed...
RESULTS AND DISCUSSION

Method A: Simultaneous Equation Method
Figure 3-6 explains overlain spectra for CEF and LZD which absorb at each other’s λ max. Analytical wavelengths CEF and LZD were 277 nm and 257 nm selected respectively. So equations 1 and 2 were directly utilized for the simultaneous estimation of CEF and LZD in sample solution.

Method B: Q-absorbance ratio method
In Q-absorbance ratio method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer’s law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 272 nm (isoabsorptive point) and 257 nm (λ-max of LZD) at which the calibration curves were prepared for both the drugs.

Method C: First Derivative Spectroscopy Method
In contrast to zero-order spectra, first derivative spectra show more resolution in terms of zero crossing points shown in Figure 7-9 explains first order derivative spectra for CEF and LZD. At 256 nm, CEF having zero crossing point and LZD can be determined. At 275 nm, CEF having zero crossing point and LZD can be determined. Table 1, Table 2 and Table 3 exhibits results of assay, results of accuracy studies and summary of various validation parameters of all methods respectively.

CONCLUSION
Three spectrophotometric methods (Simultaneous equation method, Q-absorption ratio method and first derivative spectrophotometry) were developed for simultaneous estimation of CEF and LZD in their combined pharmaceutical formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of CEF and LZD in formulation. The one-way ANOVA results show that there is no significant difference between assay results obtained from these three methods. So the proposed methods can be used in routine analysis of CEF and LZD with relatively less expensive and simple to operate instrumentation.

ACKNOWLEDGMENT
We are thankful to Bharat Parenterals ltd, Vadodara and Alembic Pharmaceuticals ltd, Vadodara for providing gift sample of CEF and LZD, respectively. We also thank Department of Quality Assurance, Pioneer Pharmacy Degree College, Vadodara for their support and guidance.

REFERENCES