Q-ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CEPFODOXIME PROXETIL AND LEVOFLOXACIN HEMIHYDRATE IN THEIR COMBINED DOSAGE FORM

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Cefpodoxime proxetil and Levofloxacin hemihydrate in combined dosage form. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the \( \lambda_{\text{max}} \) of one of the two components. Cefpodoxime proxetil and Levofloxacin hemihydrate show an isoabsorptive point at 273 nm in methanol. The second wavelength used is 300 nm, which is the \( \lambda_{\text{max}} \) of Levofloxacin hemihydrate in methanol. The linearity was obtained in the concentration range of 2-10 \( \mu \)g/ml for Cefpodoxime proxetil and 2.5-10.5 \( \mu \)g/ml Levofloxacin hemihydrate. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the \( \lambda_{\text{max}} \) of Levofloxacin hemihydrate. The method was successfully applied to pharmaceutical dosage form because of no interference. The results of analysis have been validated by recovery studies.

KEY WORDS

Cefpodoxime proxetil, Levofloxacin hemihydrate, absorbance ratio method, isoabsorptive point.

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INTRODUCTION

Cefpodoxime proxetil is [1(isopropoxycarbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-(Z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate], prodrug of Cefpodoxime. It is a third generation cephalosporin antibiotic. It binds to penicillin binding proteins (transpeptidases, endopeptidases, and carboxypeptidases) and inhibits cell wall biosynthesis in both gram positive and gram negative bacteria. They inhibit transpeptidases so that cross linking does not occur and thus prevents synthesis of bacterial cell wall.

Levofloxacin hemihydrate is [(S)-9-fluoro-2, 3-dihydro-3-methyl-1O-(4-methylpiperazin-l-yl)-7-oxo-7H-pyrido [l, .2, 3-de]-1, 4-benzoxazine-6-carboxylic acid hemihydrate]. It is Third generation fluoroquinolone antibiotic. It inhibits the replication of bacterial DNA by interfering with the action of DNA gyrase (topoisomerase II) and during bacterial growth and reproduction. Levofloxacin is twice as active as its isomer Ofloxacin.

The therapeutic importance of these two compounds justifies establishing analytical methods for its determination in bulk and pharmaceutical formulations.

The chemical structures of Cefpodoxime Proxetil and Levofloxacin Hemihydrate are shown in Figure 1 (A), (B).

![Chemical structure of Cefpodoxime Proxetil](image1)

![Chemical structure of Levofloxacin Hemihydrate](image2)

Figure 1: Chemical structure of (A) Cefpodoxime Proxetil and (B) Levofloxacin Hemihydrate.

Cefpodoxime proxetil is official in IP and USP. In IP’10 and USP’07 describe liquid chromatography for its estimation. Levofloxacin hemihydrate official in IP’10 describe potentiometry method for its estimation. So many methods like UV, HPLC, RP-HPLC available for estimation of CPD and LVX individually and in combination with other drugs.

According to detailed survey of analytical literature not even a single analytical procedure describes a simple and satisfactory UV spectrophotometric method for simultaneous determination of Cefpodoxime proxetil and Levofloxacin hemihydrate in their combined dosage forms. So the objective of this work was to develop simple, precise and rapid
spectrophotometric methods for combination drug products containing Cefpodoxime proxetil and Levofloxacin hemihydrate.

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

Cefpodoxime proxetil (CPD) and Levofloxacin hemihydrate (LVX) bulk powder was gifted by FDC Ltd. and Cipla Ltd., respectively. The commercial fixed dose combination product (GLEVOPOD) was procured from the local market. Methanol AR Grade was procured from Ranbaxy, India.

Preparation of Standard Stock solution of CPD and LVX:

Accurately weighed quantity 100 mg of CPD and LVX 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 CPD and LVX:

100 μg/ml of CPD and LVX 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, 4, 6, 8 and 10 μg/ml for CPD and 2.5, 4.5, 6.5, 8.5 and 10.5 μg/ml for LVX.

Selection of analytical wavelength:

From working standard solution of CPD (100 μg/ml) and LVX (100 μg/ml) prepare 10 μg/ml for CPD and LVX both. The scanning for solution of CPD and LVX were carried out in the range of 200-400 nm against using methanol as a blank. The maximum absorption (λmax) of LVX was found at 300 nm and iso-absorptive point at 273 nm. Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths.

Preparation of calibration curve:

Standard solutions of CPD in the concentration range of 2 to 10 μg/ml obtained by transferring (0.2, 0.4, 0.6, 0.8 and 1.0 ml) of CPD stock solution (100 μg/ml) to the series of 10 ml volumetric flasks and standard solutions of LVX in the concentration range of 2.5 to 10 μg/ml were obtained by transferring (0.25, 0.45, 0.65, 0.85 and 1.05 ml ) of LVX stock 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. The absorbances were plotted against the respective concentrations to obtain the calibration curves.
METHODOLOGY

Absorption ratio method uses the ratio of absorptions of two selected wavelength, one of which is iso-absorptive point and other being the \( \lambda_{\text{max}} \) of one of the two components. From the overlain spectra of two drugs (as shown in figure 2), it shows that CPD and LVX having iso-absorptive point at 273 nm. The second wavelength used is 300 nm, which is the \( \lambda_{\text{max}} \) of LVX. Working standard solutions having concentration 2, 4, 6, 8, and 10 \( \mu \text{g/ml} \) for CPD and 2.5, 4.5, 6.5, 8.5 and 10.5 \( \mu \text{g/ml} \) for LVX were prepared and the absorbance at 273 nm (iso-absorptive point) and 300 nm (\( \lambda_{\text{max}} \) of LVX) were measured and absorptivity coefficient were calculated using calibrations curve.

A set of two equations were framed using the mean absorptivity.

\[
\begin{align*}
Q_x &= \frac{Q_m - Q_y}{Q_x - Q_y} \cdot \frac{A_1}{a_{x_1}} \\
Q_y &= \frac{Q_m - Q_x}{Q_y - Q_x} \cdot \frac{A_1}{a_{y_1}} \\
Q_m &= \frac{\text{Absorbance of sample solution at 300nm}}{\text{Absorbance of sample solution at 273nm}} \\
Q_x &= \frac{\text{Absorptivity of CPD at 300nm}}{\text{Absorptivity of CPD at 273nm}} \\
Q_y &= \frac{\text{Absorptivity of LVX at 300nm}}{\text{Absorptivity of LVX at 273nm}}
\end{align*}
\]

Where, \( Q_x \) and \( Q_y \) are value of CPD and LVX respectively, \( a_{x_1} \) and \( a_{y_1} \) are absorptivity value at iso-absorptive point for CPD and LVX

RESULT AND DISCUSSION

Validation parameters:

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

Linearity:

The calibration curves were plotted over a concentration range of 2-10 \( \mu \text{g/ml} \) for CPD and 2.5-10.5 \( \mu \text{g/ml} \) for LVX. Accurately measured standard stock solutions of CPD (0.2, 0.4, 0.6, 0.8, 1.0 ml) and LVX (0.25, 0.45, 0.65, 0.85 and 1.05 ml ) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbances of solution were then measured at 273 nm and 300 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated [Table-4]
Table-4: Regression Characteristics:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CPD</th>
<th>LVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>273</td>
<td>300</td>
</tr>
<tr>
<td>Linearity (μg/ml)</td>
<td>2-10</td>
<td>2-10</td>
</tr>
<tr>
<td>Regression Equation</td>
<td>$y = 0.0203x + 0.0111$</td>
<td>$y = 0.0111x + 0.0154$</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0203</td>
<td>0.0111</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.9986</td>
<td>0.9985</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0111</td>
<td>0.0154</td>
</tr>
</tbody>
</table>

Figure 2: Overlain Spectrum of Cefpodoxime proxetil and Levofloxacin hemihydrates showing iso-absorptive point in Methanol.

Precision:

The precision of the instrument was checked by repeated scanning and measurement of the absorbances of solutions ($n = 6$) of CPD and LVX without changing the parameters of the proposed method. The intraday and inter-day precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3
different days over a period of one week for 3 different concentrations of standard solutions of CPD and LVX. The results of the precision study are showed in Table-5.

Table 5: Validation Parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CPD</th>
<th>LVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>273, 300</td>
<td>273, 300</td>
</tr>
<tr>
<td>Repeatability (%RSD)</td>
<td>1.3896, 0.9036</td>
<td>0.2357, 0.2357</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day</td>
<td>1.3221-1.7416</td>
<td>1.5626-1.9957</td>
</tr>
<tr>
<td>Inter-day</td>
<td>0.8875-1.8802</td>
<td>1.5801-1.9674</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>0.0221, 0.0327</td>
<td>0.0192, 0.0042</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>0.0671, 0.0991</td>
<td>0.0583, 0.0821</td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.58-100.93</td>
<td>99.63-100.44</td>
</tr>
<tr>
<td>Assay (mean±SD)</td>
<td>99.52±0.0280</td>
<td>100.23±0.0242</td>
</tr>
</tbody>
</table>

**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.

\[
LOD = 3.3 \times \frac{\sigma}{S} \quad \text{……… (3)}
\]

\[
LOQ = 10 \times \frac{\sigma}{S} \quad \text{……… (4)}
\]

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.

**Recovery Studies (Accuracy):**

The accuracy of the method was determined by calculating the recoveries of CPD and LVX by the standard addition method. Known amounts of standard solutions of CPD and LVX were at added at 50, 100 and 150 % level to pre-quantified sample solutions of CPD and LVX and absorbance were determined at 273 nm and 300 nm set of 3 replicates. The mean %
recovery was 99.58–100.90 % and 99.63–100.44 % for Cefpodoxime proxetil and Levofloxacin hemihydrate respectively [Table-1 and 2]

Table 1: Result of Recovery Studies for CPD.

<table>
<thead>
<tr>
<th>Amount of CPD in sample (μg/ml)</th>
<th>Amount of Std CPD added (μg/ml)</th>
<th>Total amount of CPD (μg/ml)</th>
<th>Total amount of CPD found (μg/ml) Mean* ± SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>3.2</td>
<td>7.2</td>
<td>7.17±0.0170</td>
<td>99.58</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8</td>
<td>7.98±0.0244</td>
<td>99.75</td>
</tr>
<tr>
<td>4</td>
<td>4.8</td>
<td>8.8</td>
<td>8.88±0.0374</td>
<td>100.90</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]

Table 2: Result of Recovery Studies for LVX.

<table>
<thead>
<tr>
<th>Amount of CPD in sample (μg/ml)</th>
<th>Amount of Std LVX added (μg/ml)</th>
<th>Total amount of LVX (μg/ml)</th>
<th>Total amount of LVX found (μg/ml) Mean* ± SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>9</td>
<td>9.04±0.0287</td>
<td>100.44</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10</td>
<td>9.99±0.0205</td>
<td>99.90</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>11</td>
<td>10.96±0.0250</td>
<td>99.63</td>
</tr>
</tbody>
</table>

[*=mean value of 3 determination]

Estimation of CPD and LVX in Pharmaceutical tablet Dosage form:

Twenty tablets were weighed and finely powdered. The powder equivalent to 40 mg CPD and 50 mg LVX was accurately weighed and transferred to volumetric flask of 100 ml capacity contains 25 ml of the methanol and sonicated it for 5 min. The flask was shaken and volume was made up to the mark with methanol. This solution was carefully filtered through what man filter paper (No. 41). Aliquot (0.1 ml) was pipette out and transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark with methanol to give a solution containing 4.0 µg/ml CPD and 5.0 µg/ml LVX. The absorbance of sample solution was measured at 273 nm and 300 nm against blank. The content of CPD and LVX
in tablet was calculated using two framed simultaneous equations. Results of analysis was done on marketed formulation and expressed in Table 3.

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>CPD</td>
<td>LVX</td>
</tr>
<tr>
<td>GLEVOPOD</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>100.42±0.0338</td>
<td>99.64±0.0178</td>
</tr>
</tbody>
</table>

[*=mean value of 6 determination]

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 par with ICH guidelines.

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REFERENCES


