SPECTROPHOTOMETRIC ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE AND CEPFODOXIME PROXETIL IN TABLET

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ABSTRACT

Objective: The present study aims to develop simple, precise and accurate methods for the estimation of levofloxacin hemihydrate and cefpodoxime proxetil by simple UV method [Absorbance ratio method] in pure and tablet dosage form.

Methods: A simultaneous determination of levofloxacin hemihydrate and cefpodoxime proxetil by absorbance ratio spectrophotometric method has been developed in combined tablet dosage form. The method is based on the measurements of absorbance at isoabsorptive point. The absorbance measurements were made six times for the formulas at 266 nm, 295.4 nm. The amounts of levofloxacin hemihydrate and cefpodoxime proxetil were found by constructing Q-analysis equation method, from the Q-analysis equation to found the amount of sample.

Results: The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.87 ± 0.2869 for Levofloxacin hemihydrate and 100.26 ± 0.1418 for Cefpodoxime Proxetil. The average %RSD values of Levofoxacin hemihydrate and Cefpodoxime Proxetil was found to be 0.2872 and 0.1414, respectively. The low % RSD value of drugs indicates that this method was accurate.

Conclusion: The wavelengths 266nm and 295.4nm were selected for the estimation of Levofloxacin hemihydrate and Cefpodoxime proxetil respectively. In this case 266 is the isobestic point for Levofloxacin hemihydrate and Cefpodoxime proxetil. The percentage label claim present in tablets was found to be 100.26 ± 0.9483 and 100.28 ± 1.9449 for Levofloxacin hemihydrate and Cefpodoxime proxetil respectively. The percentage recovery was found to be in the range of 99.87 ± 0.2869 for Levofloxacin hemihydrate and 100.26 ± 0.1418 for Cefpodoxime proxetil.

INTRODUCTION

Levofloxacin hemihydrate chemically [5(s)-9 fluoro 2,3- dihydro- 3-methyl-10-(4-methyl-1-piperazinyl) 7-oxo 7H pyridol [1,2,3-de]-1,4 benzoxazin-6-carboxylic acid] which is an antibacterial mainly used for Urinary Tract Infection. Cefpodoxime proxetil (LEVO) Chemically (6R-(6R-[6R-7α,7β(Z)])-7-[(2-amino 4thiozoyl) (methoxy imino) acetyl] amino)-3-[methoxy -methyl]- 8- oxo 5-thia-1-azabicyclo [4;2;0]oct-2-en e -2-carboxylic acid 1-[(1-methyl ethoxy )carbonyl]oxy)ethyl ester [1-3]. Levo and CEFPO are a newer combination of tablet dosage form which is used for the treatment of severe bacterial infections.

Literature survey reveals that numerous methods are available for the determinate of LEVO and CEFPO alone and in combination with other drugs.

But there no methods were reported for the estimation of these drugs in combined dosage forms without earlier separation [4-11]. The present work deals with a development of simple rapid and reproducible method for the a concurrent estimation of LEVO and CEFPO in their combined dosage form using UV spectrophotometer.

MATERIALS AND METHODS

In the present method we have attempted to assay the LEVO and CEFPO combination in tablet using the concept of iso absorbptive point. The absorption point is defined as the wavelength at which both the drugs show same absorbance. In this method the absorbance were measured at two wavelengths one being the isoabsorptive point of two components and other being the wavelength of maximum absorption of one of the two components. The combination of drug substances can be estimated simultaneously using sample spectrophotometer. As a result such method if found suitable should be inexpensive fast and industry friendly.

Instrument

An UV/VIS spectrophotometer Shimadzu model 1800 was employed for all spectroscopic measurements with 1cm matched quartz cells. LEVO & CEFO were obtained commercially.

Selection of solvent

The solubility of drugs were carried out using various polar to non polar solvents. The Solubility profiles of the drugs were determined as per the standards. The common solvent was found to be methanol. For the analysis of levofloxacin hemihydrate and cefpodoxime proxetil by the proposed method, methanol were chosen as solvent for Spectrophotometry, and it was selected on account of its ready availability, cost factor, solubility, and stability factor and cut off wavelength.

Preparation of standard stock solution of Levofloxacin hemihydrate

Pure raw material of levofloxacin hemihydrate 25 mg was accurately weighed and transferred to a 100 ml standard flask and dissolved in methanol and produce 100 ml with methanol. From this 10 mL of the solution was transferred in to the 100 ml volumetric flask and made up to required volume with methanol to get the concentration 25 g ml-1. It is used as a working standard.

Preparation of standard stock solution of Cefpodoxime proxetil

Pure raw material of cefpodoxime proxetil 20 mg was accurately weighed transferred to a 100 ml standard flask and dissolved in methanol and produce 100 ml with methanol. From this 10 mL of the solution was transferred in to the 100 ml volumetric flask and made up to required volume with methanol to get the concentration 20 g ml-1. It is used as a working standard.

Selection of wavelengths

The selection of wavelength for the estimation of levofloxacin hemihydrate and cefpodoxime proxetil, a suitable standard solution...
to contain 10 g/ml of levofloxacin hemihydrate and cefpodoxime proxetil were prepared individually and scanned in the entire range from 200-400 nm. From the overlaid spectra, the two wavelengths 295.4 nm for levofloxacin hemihydrate and 260 nm for cefpodoxime proxetil were selected for the construction simultaneous equation method. For Absorbance ratio method, the isobestic point was selected for both the drugs from their zero order spectra’s, which is 266 nm, and other one is λ max of levofloxacin such as 295.4 nm for levofloxacin hemihydrate and 260 nm for cefpodoxime proxetil.

**Linearity**

The linearity studies for the Absorbance ratio method was performed at 266 nm, 295.4 nm in the concentration range of 2.5-15 µg/mL and other one is λ max of levofloxacin such as 295.4 nm for levofloxacin hemihydrate and 260 nm for cefpodoxime proxetil.

**Accuracy (Recovery studies)**

Performing at 266 nm, 295.40 nm in the concentration range of 2.5-15 µg/mL for Levofloxacin hemihydrate and 4-24 µg/mL for cefpodoxime proxetil.

**Preparation of calibration curve**

The calibration curves were constructed by plotting absorbance Vs concentration for absorbance ratio method. The optical characteristics and regression equation for the calibration curves are presented in Table 1.

**Validation of developed method**

**Linearity and Range**

From the calibration graphs plotted, levofloxacin hemihydrate and cefpodoxime proxetil shows the linearity between 2.5-15 µg/mL, 4-24 µg/mL for Absorbance ratio method.

### Table 1: Optical characteristics and regression equation for the calibration curves

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters Values</th>
<th>LEVO</th>
<th>CEFPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>λ- max(nm)</td>
<td>295.4</td>
<td>260</td>
</tr>
<tr>
<td>2.</td>
<td>Beer’s law limit(µg/ml)</td>
<td>2.5-15µg/ml</td>
<td>4-24µg/ml</td>
</tr>
<tr>
<td>3.</td>
<td>Molar absorptivity</td>
<td>1.397X10⁴</td>
<td>1.657X10⁴</td>
</tr>
<tr>
<td>4.</td>
<td>Sandell's sensitivity (µg/cm²/0.001AV)</td>
<td>77.8</td>
<td>0.0007748</td>
</tr>
<tr>
<td>5.</td>
<td>Regression equation (A.Slope(b))</td>
<td>0.0778</td>
<td>0.19766</td>
</tr>
<tr>
<td></td>
<td>Intercept(a)</td>
<td>0.00393</td>
<td>0.005714</td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient(r)</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

A= a+bc (where ‘c’ is the concentration in µg/mL).

**Iso Absorptive method**

For the present study two brands of commercial tablets designated as Tablet A and Tablet B each containing 250mg of LEVO and 200mg of CEFPO were procured from open market. Twenty tablets of each brand were weighed and crushed to fine powder. Next, powder containing 25mg of LEVO and 20mg of CEFPO was weighed and transferred and dissolved in methanol. The solution was filtered through whatmann filter paper No.41 and diluted to 100ml with methanol. The aliquots of solution was diluted to give final dilution of concentration 12.5mg/mL of LEVO and 10mg/cm CEFPO. The absorption of the solution was next measured at 295.4 nm and 260 nm. The concentration of LEVO and CEFPO in each tablet was calculated according to method of pernerwaski et al [12]. These such determination were carried out for each brand and the mean is calculated Table 2.

### Table 2: Results of Commercial tablet formulation analysis

<table>
<thead>
<tr>
<th>Tablet sample</th>
<th>Label Claim % of label claim</th>
<th>Standard Coefficient of (mg/tab) estimated* estimated</th>
<th>Deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet A</td>
<td>LEVO 250mg</td>
<td>LEVO 200.28</td>
<td>0.9483</td>
<td>0.9457</td>
</tr>
<tr>
<td></td>
<td>CEFPO 200mg</td>
<td>CEFPO 100.03</td>
<td>0.8076</td>
<td>0.8059</td>
</tr>
<tr>
<td>Tablet B</td>
<td>LEVO 250mg</td>
<td>LEVO 250.7</td>
<td>0.8043</td>
<td>0.8027</td>
</tr>
<tr>
<td></td>
<td>CEFPO 200mg</td>
<td>CEFPO 250.3</td>
<td>0.8076</td>
<td>0.8059</td>
</tr>
</tbody>
</table>

*Average of ix Determinations.

The developed method was confirmed for ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts, different sources of reagents and so on. In the present work it was confirmed by different analysts. The low % RSD value indicates that the developed method was more rugged. The results were shown in Table 3.

### Table 3: ruggedness studies of 50% pre analysed Formulation of proposed method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percentage</th>
<th>Condition</th>
<th>Analyst 1</th>
<th>Analyst 2</th>
<th>% S.D</th>
<th>% R.S.D</th>
<th>S.E</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEVO</td>
<td>80</td>
<td>Analyst 1</td>
<td>0.3579</td>
<td>0.3575</td>
<td>0.3579</td>
<td>0.3575</td>
<td>0.0397</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Analyst 2</td>
<td>0.4033</td>
<td>0.4027</td>
<td>0.4033</td>
<td>0.4027</td>
<td>0.0375</td>
<td></td>
</tr>
<tr>
<td>CEFPO</td>
<td>80</td>
<td>Analyst 1</td>
<td>0.0346</td>
<td>0.0348</td>
<td>0.0346</td>
<td>0.0348</td>
<td>0.0038</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Analyst 2</td>
<td>0.6051</td>
<td>0.6038</td>
<td>0.6051</td>
<td>0.6038</td>
<td>0.0593</td>
<td></td>
</tr>
</tbody>
</table>

**Accuracy (Recovery studies)**

Accuracy of the method was confirmed by recovery studies.

**Precision**

The repeatability of the method was confirmed by the analysis of tablets repeated for 6 times with the same concentration. The amount of each drug present in the tablets were calculated. The percentage RSD was calculated. The amount of drugs was determined and the percentage RSD was calculated.

**LOD and LOQ**

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of response.
The proposed method for simultaneous estimation of LEVO & CEFPO in combined dosage forms was found to be simple, accurate, economical and rapid.

The correlation coefficient for both the drugs was found to be around 0.9999. This indicates that both the drugs obey Beer’s law and they were linear at the selected concentration range.

The concentration of solution containing 12.5g mL⁻¹ of Levofloxacin hemihydrates and 10g mL⁻¹ of Cefpodoxime Proxetil was prepared and the area of the solutions was measured at their respective wavelength ranges. The percentage purity of tablets was found to be 100.26±0.9483 and 100.28±1.9449 for Levofloxacin hemihydrate and Cefpodoxime Proxetil, respectively. The amount present in tablets was in good concord with the label claim and the % RSD values were found to be 0.9457 and 1.9394 for Levofloxacin hemihydrate and Cefpodoxime Proxetil, respectively. The low % RSD value indicates that the method was highly precise.

The developed method was validated for ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts, different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value 80%, 100%, 120% by analyst 1 and analyst 2 was found to be 0.3575, 1.4515, 0.8402 and 0.4027, 0.4276, 0.2019 for Levofloxacin hemihydrate and 0.0348, 0.4640, 0.1995 and 0.06.0308, 0.0870, 0.7029 for Cefpodoxime Proxetil, respectively. The low % RSD value indicates that the developed method was more rugged.

The accuracy of the method was performed by recovery studies. To the 50% pre analysed formulations, a known quantity of Levofloxacin hemihydrate and Cefpodoxime Proxetil raw material solutions were added at three different concentration levels. The recovery was calculated. The percentage recovery was found to be in the range of 99.87±0.2869 for Levofloxacin hemihydrate and 100.26±0.1418 for Cefpodoxime Proxetil.

The average %RSD values of Levofloxacin hemihydrate and Cefpodoxime Proxetil was found to be 0.2872 and 0.1414, respectively. The low % RSD value indicates that the method was accurate.

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The average %RSD values of Levofloxacin hemihydrate and Cefpodoxime Proxetil was found to be 0.2872 and 0.1414, respectively. The low % RSD value indicates that the method was accurate.

RESULTS AND DISCUSSION

The proposed method was accurate.