Research article

Development and validation of UV spectrophotometric estimation of lisinopril dihydrate in bulk and tablet dosage form using area under curve method

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Abstract
A simple, precise, accurate, and economical UV visible spectrophotometric method has been developed for estimation of Lisinopril Dihydrate drug by AUC method. The standard and sample solutions were prepared by using distilled water and Methanol 70:30 as a solvent. Quantitative determination of the drug was performed at wavelength range 210-220 nm. The linearity was established over the concentration range of 10-35 μg/ml for Lisinopril Dihydrate with correlation coefficient value of 0.999. Precision studies showed that % relative standard deviation was within range of acceptable limits. The mean percentage recovery was found to be 99.610%. The proposed method has been validated as per ICH guidelines.

Key words: Lisinopril Dihydrate, UV visible spectrophotometry, AUC, Method Validation.

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Introduction
Lisinopril (Figure 1) is an orally bioavailable, long-acting angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity. Lisinopril, a synthetic peptide derivative, specifically and competitively inhibits ACE, which results in a decrease in the production of the potent vasoconstrictor angiotensin II and, so, diminished vasopressor activity [1-3]. Literature survey reveals the availability of several methods by using various mixture but no method was available on the mixture of distilled water and Methanol 70:30 which was an unique method with better results [4-6].
Materials and methods

Apparatus and instrumentation
A Shimadzu 1800 UV/VIS double beam spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath. Calibrated volumetric glassware (Borosil®) was used for the validation study.

Materials
Reference standard of Lisinopril Dihydrate API was supplied as gift sample by Wochardt Pharmaceutical Ltd., Aurangabad. Tablet sample with label claim 10 mg per tablet were purchased from local market.

Method development

Determination of Wavelength Range
For the selection of analytical wavelength range for area under curve method, 20 μg/ml solution of Lisinopril Dihydrate was scanned in the spectrum mode from 400 nm to 200 nm against solution (distilled water and Methanol 70:30) as blank. Wavelength range was selected around wavelength maxima (216 nm). Different working standards were prepared between 10-35 μg/ml. Various wavelength range were tried and final wavelength range between 210-220 nm was selected on the basis of linear relationship between area and corresponding concentration (Figure 2).

Area under curve (Area calculation)
Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 210 to 220 nm.

Preparation of standard solution
The standard stock solution of Lisinopril Dihydrate was prepared by accurately weighing & transferring, 10 mg of API to 100 ml of volumetric flask. The drug was dissolved with sonication in 50 ml of mixture(35 ml distilled water and 15 ml Methanol) volume was made up to the mark by using Mixture. Then from that 0.2ml was taken and added to 10ml volumetric flask and make up with distilled water to get final standard stock solution (20 μg/ml) and other dilution are made with distilled water to obtain 10-35 μg/ml Lisinopril Dihydrate solutions.

Calibration curve for Lisinopril Dihydrate
The dilutions were made from Standard Stock solution to get concentration of 10, 15, 20, 25, 30, 35 μg/ml respectively. These solutions were scanned from 400 to 200 nm and area under curve (AUC) values was integrated in the range of 210-220 nm. The calibration curve was plotted between areas under curve values against concentration (Figure 3).

Table 1. Concentration vs Absorbance

<table>
<thead>
<tr>
<th>Concentration in μg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.29</td>
</tr>
<tr>
<td>15</td>
<td>0.50</td>
</tr>
<tr>
<td>20</td>
<td>0.68</td>
</tr>
<tr>
<td>25</td>
<td>0.90</td>
</tr>
<tr>
<td>30</td>
<td>1.10</td>
</tr>
<tr>
<td>35</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Figure 2. UV AUC spectrum of Lisinopril Dihydrate (20μg/ml).
Assay of tablet formulation
Twenty tablets each containing 10 mg of Lisinopril Dihydrate were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Lisinopril Dihydrate was transferred in 100 ml of volumetric flask. A 50 ml of solution (35 distilled water and 15 ml Methanol) was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with Mixture. The solution was filtered using Whatmann filter paper no. 41, first 5 ml of filtrate was discarded. This solution was further diluted to obtain 20 μg/mL solution with Mixture, subjected for UV analysis using distilled water as blank. This procedure was repeated three times (Table 2).

Table 2. Assay of tablet dosage form.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample Solution Concentration (μg/ml)</th>
<th>Amount found (%)</th>
<th>Mean % found</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>101.13</td>
<td>99.61</td>
<td>1.464627</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>98.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>99.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Accuracy
The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 20 μg/ml standard solution. Area under curve (AUC) was measured in wavelength range 210-220 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level as shown in table 3.

Table 3. Accuracy results for Lisinopril Dihydrate.

<table>
<thead>
<tr>
<th>Accuracy level</th>
<th>Sample conc (μg/ml)</th>
<th>Std. Conc.</th>
<th>Total amount Added (μg/ml)</th>
<th>% Recovery</th>
<th>Mean %Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>10</td>
<td>08</td>
<td>18</td>
<td>98.85</td>
<td>99.51</td>
<td>1.6005</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>99.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>100.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Precision
The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions intraday precision was studied by integrating area of standard solution of 20 μg/ml concentration at six independent series in the same day. Inter-day precision studies were performed by integrating area of standard solution of 20 μg/ml concentration on three consequent days. The %RSD was calculated as shown in table 4.
**Linearity and Range**

The linearity was determined by using working standard solutions between 10-35 μg/ml. The areas under curve (AUC) of these solutions were recorded. Calibration curve of area under curve to concentration plotted on excel sheet and linear regression was performed. The correlation coefficient, regression Equation was calculated. (Figure 3)

**Table 4. Precision results for Lisinopril Dihydrate**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intra day</th>
<th>Inter day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample sol conc μg/ml</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>AUC (mean)</td>
<td>0.2415</td>
<td>0.3210</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.3415</td>
<td>0.8810</td>
</tr>
</tbody>
</table>

**Limit of Detection and Limit of Quantification**

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

\[
LOD = \frac{3.3 \sigma}{S}
\]

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

\[
LOQ = 10 \frac{\sigma}{S}
\]

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

LOD & LOQ of Lisinopril Dihydrate was found to be 0.85516 μg/ml & 2.65107 μg/ml respectively.

Five sets of known concentrations (10-35 μg/ml) were prepared and scanned. By using these spectras, regression equations were obtained. By taking average of slopes and standard deviation of y-intercept, LOD and LOQ were calculated. The values of LOD and LOQ are given in table 5.

**Result and Discussion**

The UV visible spectroscopic method for the Lisinopril Dihydrate by area under curve was found to be simple, accurate, economical and reproducible. The drug concentrations were found to be linear in the range of 10-35 μg/ml and the correlation coefficient value of 0.999 indicates that developed method was linear. For Precision the percent relative standard deviation (% RSD) was found to be 0.3415 while, intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 0.3415 and 0.8810 respectively thus the method is observed as precise. The accuracy of the method was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value is ≤ 2 indicates the accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be 0.85516 μg/ml & 2.65107 μg/ml respectively. The result of the analysis for pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The validation parameters are summarized in Table 5. The method can be used for routine
quality control analysis of Lisinopril Dihydrate in bulk and pharmaceutical formulations.

Conclusion

The UV spectroscopic AUC method for the analysis of Lisinopril Dihydrate was found to be simple, precise, and accurate, can be used for assay of bulk drug and pharmaceutical dosage formulations.

References