DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF DALFAMPRIDINE IN TABLETS

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Abstract
A simple, accurate, precise, reproducible, economic spectrophotometric method has been developed for the estimation of Dalfampridine in tablet dosage form. UV spectrophotometric method is based on measurement of absorption at maximum wavelength 247 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision. Beer’s law was obeyed in the concentration range of 2 – 10 µg/mL with correlation coefficient of 0.9965. Results of the analysis were validated statistically and by recovery study. Hence the developed and validated method can be used for estimation of dalfampridine in tablets.

Keywords: UV Spectroscopy, Dalfampridine, Development, Validation

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INTRODUCTION

Dalfampridine is a potassium channel-blocker that enhances conduction in focally demyelinated axons, improves synaptic transmission and potentiates muscle contraction. Clinically, dalfampridine has been found to improve walking in patients with multiple sclerosis. The largest scale industrial application of 4-aminopyridine is as a precursor to the drug pinacidil, which affects potassium ion channels. The IUPAC name of dalfampridine 4-Aminopyridine. The structure is of Dalfampridine is given Fig.1. It occurs as a white crystalline powder with a molecular weight of 94.11 g/mol. It is soluble in polar organic solvents such as methanol and water [1,2,3].

Spectroscopy is one of the most powerful tools for the analysis of a wide range of pharmaceutical dosage forms. If a suitable method, for specific need, is not available then it becomes essential to develop a simple, sensitive, accurate, precise and rapid method for the estimation of drug samples. Dalfampridine is not official in I.P and B.P. Literature review revealed that there are few methods based on Analysis of plasma samples was performed by LC-MS/MS and reverse phase ion-pair HPLC with UV detection [4-5].

The present work describes the development and validation of UV spectrophotometric method, which can quantify the Dalfampridine (Fig 1). An attempt was made to develop a simple, accurate, precise and rapid spectrophotometric method for the estimation of dalfampridine in tablet dosage form in distilled water. The method was validated as per International conference on Harmonization (ICH) guidelines.
MATERIALS AND METHODS

Materials

Dalfampridine was obtained as gift sample from Aurobindo pharma limited. (Hyderabad, A.P, INDIA). All chemicals used were of analytical grade. Ampyra (10 mg) tablet formulation of Dalfampridine was purchased.

Instruments

UV 1800 Shimadzu double beam spectrophotometer, wavelength accuracy ±0.5 nm and a pair of 1.0 cm matched quartz cells were used to measure absorbance of resulting solution.

Preparation of Standard Solution

Preparation of Dalfampridine standard solutions accurately weighed 10 mg of Dalfampridine was transferred into 100 ml volumetric flask. It was dissolved and diluted up to mark with methanol to obtain stock solution (100µg/mL). The standard solutions in concentration range of 2-10 µg/mL were prepared by dilutions of the stock solution with double distilled water. The determination was conducted six times at room temperature [6-10].

Preparation of Sample Solution (Assay of Marketed Formulation)

10 tablets were weighed to obtain the average tablet weight, which were then powdered. Powder equivalent to 10 mg of dalfampridine was weighed and transferred to 100 ml volumetric flask and allowed to dissolve in 70ml methanol. This mixture was sonicated for 15 min to ensure complete solubility of the drug and filtered through Whatman filter paper no. 41. The volume was made up to mark with methanol to get the solution having dalfampridine 100 µg/mL and the sample solutions were prepared with in the concentration range by using double distilled water[11-12]. The results were reported in Table 1.
Table No: 1 Assay of Dalfampridine marketed formulation (Tablet)

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>S.NO</th>
<th>Lable claim</th>
<th>Amount (mg)</th>
<th>Percentage of Drug recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPYRA</td>
<td>1</td>
<td>10</td>
<td>9.95</td>
<td>99.5</td>
<td></td>
</tr>
<tr>
<td>AMPYRA</td>
<td>2</td>
<td>10</td>
<td>10.15</td>
<td>101.5</td>
<td>1.0±0.51</td>
</tr>
<tr>
<td>AMPYRA</td>
<td>3</td>
<td>10</td>
<td>9.98</td>
<td>99.8</td>
<td></td>
</tr>
</tbody>
</table>

Scanning for $\lambda_{\text{max}}$

The standard solution of Dalfampridine was scanned in the wavelength range of 200nm - 400 nm using UV spectrophotometer. The spectrum was depicted in Figure 3.

Preparation of Calibration Graph Using UV Spectrophotometric Method

A calibration graph was constructed over a concentration range of 2-10 µg/mL. Absorbance of each solution was measured at the wavelength of 247 nm. Calibration graph was constructed for Dalfampridine by plotting concentration (µg/mL) Versus absorbance at 247 nm. The graph was depicted in Figure 2.

![Figure 2: Calibration graph of Dalfampridine](image-url)
RESULTS AND DISCUSSION

Development and Optimization

The solubility of Dalfampridine was tested in water, methanol and 0.1N NaOH. Based upon the free solubility of Dalfampridine in methanol and water, they were selected as solvent for method development of Dalfampridine estimation. The analyte was estimated at 247nm and respective UV spectrum was depicted in Figure 2.

![UV Spectrum for standard Dalfampridine](image)

Validation

The developed UV spectrophotometric method was validated for linearity range, accuracy, precision, Limit of detection, limit of quantification parameters as per ICH guidelines [13-14].

Linearity Range

The linearity was determined for Dalfampridine by plotting a calibration graph of concentration against absorbance. Dalfampridine showed linearity in the range of 2-10 µg/mL. Calibration
curve of Dalfampridine was depicted in Figure 3. The linear regression for the drug was represented in Table 3. **Table No: 2 Accuracy data (Recovery studies)**

<table>
<thead>
<tr>
<th>S. No</th>
<th>concentration of Drug in formulation(µg/ml)</th>
<th>concentration of pure drug added(µg/ml)</th>
<th>total concentration of drug (µg/ml)</th>
<th>Amount found(µg/ml)</th>
<th>drug recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>17.98</td>
<td>98.8</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>19.91</td>
<td>99.5</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>22.12</td>
<td>100.5</td>
</tr>
</tbody>
</table>

**Table No: 3 Summary of validated parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>247nm</td>
</tr>
<tr>
<td>Beer’s Linearity (µg/ml)</td>
<td>2 – 10 µg/ml</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td>262.93</td>
</tr>
<tr>
<td>Regression equation (Y = mx + c)</td>
<td>(Y = 0.0835x)</td>
</tr>
<tr>
<td>a) Slope (m)</td>
<td>0.0835</td>
</tr>
<tr>
<td>b) Correlation coefficient (r^2)</td>
<td>0.9965</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Accuracy**

Accuracy of developed method was determined by a recovery study at 3 concentration levels by replicate analysis (n=3). Standard drug solutions were added to a pre-analysed sample solution and percentage of total drug content was calculated. The results of accuracy studies were reported in Table 2.
Precision

Precision was determined by studying the repeatability and intermediate precision. The standard deviation and relative standard deviation were calculated for the drug. Repeatability was determined by six estimations of Dalfampridine 2-10 µg/mL and %RSD was calculated. The results of precision studies were reported in Table 3.

CONCLUSION

The developed UV Spectrophotometric method was found to be simple, economic, easy, accurate, precise, reproducible and highly sensitive and can be used for routine estimation of Dalfampridine in bulk and other formulations.

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