HPLC method development of rivastigmine by RP-HPLC in its bulk dosage form

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ABSTRACT

A fast simple sensitive precise, accurate and reproducible RP-HPLC method was developed and validated for the analysis of rivastigmine bulk dosages form. The separation was conducted by using C-18 RP-HPLC column. Which was maintained at ambient temperature. The mobile phase consist Potassium dihydrogen phosphate buffer and acetonitrile (70/30 v/v) was delivered at a rate of 1ml/min. The analysis was detected by using UV detector at the wavelength 217nm. The method is validated for its precision, limit of quantitiation (LOQ) linearity and robustness. The method was found to be linear over the concentration range 10-100 µg/ml (r² =0.999). The retention time for rivastigmine was found to be 3.66± 0.25min. limit of quantitation of method is 0.196 µg/ml and limit of detection 0.056 µg/ml.

Key words: C-18 RP-HPLC column, acetonitrile.

INTRODUCTION

Dementia is a progressive brain dysfunction, which results in a restriction of daily activities and in most cases leads in the long term to the need for care. Alzheimer’s disease (AD) is the most Frequent type of dementia in old age [1]. Rivastigmine is chemically (−)S-N-ethyl-3-[(1-dimethylamino)ethyl]-N-methyl phenyl-carbamate hydrogen tartarate, a carbamate inhibitor of acetyl cholinesterase is used for the treatment of mild to moderate Alzheimer’s disease in adults[2]. More recent efforts have focused on augmenting cholinergic transmission by blocking the activity of cholinesterase’s that degrade ACh at the synaptic junction [3-5]. Several cholinesterase inhibitors (ChEIs) are available and have been shown, with varying degrees of efficacy, to slow the AD-associated decline in behavior, cognition, and the ability to perform activities of daily living (ADL). Four ChE-Is have been approved by the United States Food and Drug Administration (U.S. FDA) are marketed for the treatment of Alzheimer’s disease are donepezil (Aricept), rivastigmine (Exelon), galantamine (Reminyl) and tacrine (Cognex). These four agents represent different classes of ChE-Is and have different pharmacologic properties beyond inhibition of Acetylcholinesterase (AChE) [6]. Literature survey revealed that a few high performance liquid chromatography (HPLC) methods reported are applicable for analysis of Rivastigmine in body fluids [7-13]. A Spectrofluorimetric method has been developed for the determination of Rivastigmine in pharmaceuticals[14].This paper describes an accurate, fast, simple, precise and sensitive method for the analysis of rivastigmine by using reverse phase high performance liquid chromatography(RP-HPLC) [15,16]. The proposed method is optimized and validated as per the international conference on harmonization (ICH) guideline[17,18].
MATERIALS AND METHODS

Instrumentation:
Quaternary isocratic HPLC (Younglin HPLC YL9000 series) with YL 9110 Pump and with “autochrome 3000” software and UV-Visible detector YL9120, electronic balance (shimadzu) was used for weighing the samples, were injected on to HPLC system using Hamilton micro syringes.

Reagents and chemicals:
Rivastigmine was obtained from FDC limited Goa and acetonitrile and water employed for the preparation of mobile phases were of HPLC grade (Qualigens fine chemicals Mumbai). All the other chemicals and solvents viz. Phosphate buffer, Acetonitrile are of ambient grade.

Chromatographic conditions:
The mobile phase for the proposed method potassium di hydrogen ortho phosphate and acetonitrile (70:30v/v) was filtered through a 0.45-µm membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column inerstil C 18 column (250×4.6mm) at flow rate 1.0ml/min. The run time was set at 5 min the column temperature was maintained at room temperature. Prior to injecting the drug solution in to the column, the column was equilibrated for at least 1 hour with the mobile phase flowing through the system. The eluent was monitored at 217nm. The data was stored and analyzed with the software “autochrome-3000” (youngling).

Selection of mobile phase:
The solution of Rivastigmine was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally Phosphate buffer and Acetonitrile (70:30v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Rivastigmine.

Preparation of mobile phase:
Mobile phase comprised of 20 mM Potassium di hydrogen ortho phosphate. (Adjusted to pH 3.0 ±0.05 with Ortho phosphoric acid), and Acetonitrile (70:30v/v), diluent (pH 7) used was water. Mobile phase was filtered through a 0.45-µm membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1.5 ml/min), which yields a column back pressure of 650-723 psi. Run time was set as 5 min, column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 217nm and data were acquired, stored and analyzed with the software “Autochro-3000” (Young Lin).

EVALUATION OF ANALYTICAL METHODS

Linearity:
Aliquots ranging from 10-100 µg/ml were prepared by suitable dilution of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for Rivastigmine, the higher concentration range was used to improve signal to noise ratio. Linearity was determined by analyzing five working standard solutions over the concentration range of 10-100µg/ml for Rivastigmine.

Precision:
Five sets of aliquots with same concentration (50 µg/ml) were prepared and these solutions were analyzed to record any intra and inter day variations in the results. The results obtained for Intra and inter day variations

Limit of detection LOD:
The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula,

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

Where, \(\sigma\) = standard deviation of the response 
\(S\) = slope of the calibration curve
Limit of Quantification (LOQ)
The limit quantification is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula,

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

Where, \( \sigma \) = standard deviation of the response
\( S \) = slope of calibration curve

RESULT AND DISCUSSION

Checking of resolution of drug and materials:
The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of Rivastigmine was injected to get the chromatogram. The retention time for Rivastigmine was found to be 3.66 ± .25 min. It is shown in the (Table.1)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Drug</th>
<th>RT (min)</th>
<th>Peak Area</th>
<th>Height</th>
<th>Plates</th>
<th>HETP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rivastigmine</td>
<td>3.66</td>
<td>3745895</td>
<td>43314</td>
<td>56481</td>
<td>0.0562</td>
</tr>
</tbody>
</table>

Table 1. Resolution of drug material

Linearity:
The data of the peak area of the Drug Vs drug concentration were evaluated by linear regression analysis as shown in the (Table.2) and calibration curve obtained after plotting drug concentration Vs area shown in the (fig.1). Linear regression analysis demonstrated that chromatograph response for the drug was highly linear (\( r^2 \leq 0.999 \)) in the studied concentration range of 10-100µ/ml. A typical chromatogram of rivastigmine (50µg/ml)shown in (fig 2)

![Fig.1. A typical chromatogram for rivastigmine (50µg/ml)](image-url)
Fig 2: Calibration curve of Rivastigmine

Table: 2 Linearity of rivastigmine

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Retention Time(min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>3.667</td>
<td>795623</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>3.598</td>
<td>2145630</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>3.669</td>
<td>3745895</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>3.667</td>
<td>5563248</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>3.662</td>
<td>7123564</td>
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</table>

Precision:
The result depicted in the table3a,3b indicated that the given method has sufficient precision as indicated by the corresponding values of %RDS ranging 0.12 for inter day studies respectively. The values of %RDS for both the studies are well below 1.0% constructing adequate precision.

Table. 3a.Intra-day Precision for Rivastigmine

<table>
<thead>
<tr>
<th>Concentration(µg/ml)</th>
<th>Peak Area</th>
<th>Mean(n=5)</th>
<th>S.D</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3756489</td>
<td>28226.89</td>
<td>27226.89</td>
<td>0.12</td>
</tr>
<tr>
<td>50</td>
<td>3812456</td>
<td>28226.89</td>
<td>27226.89</td>
<td>0.12</td>
</tr>
<tr>
<td>50</td>
<td>3756231</td>
<td>28226.89</td>
<td>27226.89</td>
<td>0.12</td>
</tr>
<tr>
<td>50</td>
<td>3802345</td>
<td>28226.89</td>
<td>27226.89</td>
<td>0.12</td>
</tr>
<tr>
<td>50</td>
<td>3756123</td>
<td>28226.89</td>
<td>27226.89</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table. 3b.Inter-day Precision for Rivastigmine

<table>
<thead>
<tr>
<th>Concentration(µg/ml)</th>
<th>Peak Area</th>
<th>Mean(n=5)</th>
<th>S.D</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3716489</td>
<td>3856231</td>
<td>28226.89</td>
<td>0.15</td>
</tr>
<tr>
<td>50</td>
<td>3712456</td>
<td>3856231</td>
<td>28226.89</td>
<td>0.15</td>
</tr>
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<td>28226.89</td>
<td>0.15</td>
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<tr>
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<td>3756123</td>
<td>3856231</td>
<td>28226.89</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Limit of detection and quantification:
Standard error and slope of linear data is used to predict LOD and LOQ of rivastigmine and precision was established at the predict concentration. The result was shown in the table.4
Table 4 Limit of detection and Limit of quantification

<table>
<thead>
<tr>
<th>Limit of detection</th>
<th>Limit of quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.056 µg/ml</td>
<td>0.196 µg/ml</td>
</tr>
</tbody>
</table>

CONCLUSION

The developed method was validated in terms of precision, linearity, limit of detection, and limit of quantification. A good linear relationship was observed from rivastigmine in the concentration range of 10-100 µg/ml. The co-relation co-efficient for rivastigmine was found to be as 0.999. The intra and inter day precision was good enough to indicate that the developed method is precise and reproducible. This demonstrated that the current developed RP-HPLC method is simple, linear, and precise.

Acknowledgment

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REFERENCES