ABSTRACT

To develop a selective and sensitive, new, simple, precise & accurate method for Donepezil hydrochloride analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated. Chromatography was carried out using a C18 (4.6 X 250 mm 3.5µm). A mobile phase consisting of Methanol:buffer (60:40%v/v) was pumped at an isocratic flow rate of 1ml/min. The calibration curves were linear (0.999) in the concentration ranges of 200-400 µg /mL for Donepezil HCl. The mean absolute recoveries for 50 and 150 µg /mL Donepezil HCl were 100.26 and 101.5, respectively. The lower limits of quantification were 7.5 µg/mL and limit of detection is 2.27ug/mL of Donepezil HCl and RT is 4.287. The proposed method was validated by determining sensitivity accuracy, precision, robustness stability, specificity, and selectivity and system suitability parameters.

Keywords: RP-HPLC, Validation, Donepezil.

INTRODUCTION

DONEPEZIL HCl \(1^2\), 3-dihydro 5, 6, dimethoxy2-[1-phenylmethyl] -piperidynyl]-H Indane-1-one HCl \(2^3\) (Fig No: 1) Donepezil is a specific noncompetitive, reversible inhibitor of acetylcholinesterase (AChE) \(3^4\), 5, and appears to exert its therapeutic effect by enhancing cholinergic function \(4^6\). \(5^6\) As the dementia progresses, fewer cholinergic neurons are thought to remain functionally intact, and the effects of Donepezil may be lessened \(5^5\). Donepezil exhibits a relatively high degree of selectivity for neuronal Ach\(2^6\), 5, 6, at relevant clinical doses, it has only weak inhibitory effects on butyrylcholinesterase (pseudo cholinesterase), an enzyme that is widely distributed in plasma and peripheral tissues \(6^,\) -\(3\). Animal studies have shown that Donepezil exhibits tissue selectivity; it significantly inhibits Ach\(3^6\) in the brain but causes little inhibition of Ach\(1\) in smooth, striated, or cardiac muscle \(5^7\). Donepezil inhibition of AChE in red blood cells corresponds closely to its effect at synapses in the central nervous system (CNS) \(3^,\) 6, 7, and 9. AChE inhibition in red blood cells has been used as an indicator of the clinical effectiveness of Donepezil in Alzheimer’s disease patients \(3^,\).

[Chemical structure of Donepezil HCl]

The most commonly used techniques for the determination of Donepezil hydrochloride in biological fluids and tablets are high-performance liquid chromatography (HPLC) equipped with an ultraviolet (UV) \(8^9\), 10, fluorescence (FL) \(12^\), 13 or mass spectrometric (MS) detector \(14^\). Solvent extraction spectrophotometry \(15^6\) spectrophorimetry \(17^6\) and colorimetry \(18^6\) have also been used. In the light of the above literature a simple spectrophotometric method for the determination of Donepezil hydrochloride has been developed. The proposed method offers several advantages, because HPLC \(9^15\) methods are very sensitive but need sophisticated instrumentation and expert hands. Extraction\(16\) procedure always not quantitative. The present procedure neither requires any extraction nor pH maintenance nor any elaborate equipment and the method is less time consuming. UV-visible spectrophotometer with 10mm matched quartz cells were used for absorbance measurement.

MATERIAL AND METHODS

Instruments Used


Materials and reagents

Acetonitrile HPLC Grade Methanol HPLC Grade Potassium dihydrogen phosphate AR Grade/Merck Water Milli pore water

Solubility

According to literature, Donepezil hydrochloride is freely soluble in chloroform, glacial acetic acid water and ethanol. And it was checked for different dilutions of methanol for solubility of Donepezil hydrochloride. Finally methanol was chosen as solvent for present work.

Selection of wavelength: (\(\lambda_{max}\))

An ideal wavelength is one that uses good response for the drugs to be detected. Donepezil hydrochloride in diluents the spectra was scanned on UV Visible spectrophotometer meter in the range of 200nm to 300nm against Diluent as blank. The simultaneous estimation of Donepezil HCl was found to be 230nm. From the UV Visible spectrophotometric results, the detection wavelength of 230nm was selected because at this wavelength they showed maximum absorbance (fig no.2). Also at 230nm the chromatogram was observed in PDA detector which was having very high absorbance compared to 260nm. So the chromatographic condition was optimized at 230nm

Preparation of mobile phase

Preparation of phosphate buffer (0.01%) 

3.5gm of KH\(\text{PO}_4\) was dissolved in 500 ml of water and adjusted with orthophosphoric acid at pH 6. The above buffer and mobile phase was mixed in the ratio of 50: 50 and it was degassed and sonicated for about 5 min.

Standard preparation

10 mg of Donepezil hydrochloride working standard was transferred into 100 ml volumetric flask, dissolved and diluted to volume with mobile phase and mixed. Pipette out 1.2 ml of the above solution into 10 ml of volumetric flask, diluted to volume with mobile phase.

Test preparation

For estimating the tablet dosage form, 20 tablets from a batch were
randomly selected and powdered. Weigh accurately 0.7 gm of ground tablet powder (equivalent to 10 mg of Donepezil hydrochloride) transfer it in 100 ml of volumetric flask and add 100 ml of mobile phase, shake the flask on a rotator shaker for 30 min and sonicate for 15 min with intermediate shaking. Keep the solution on a rotatory shaker for 30 min at 200 rpm. Centrifuge the portion of above solution at 4000 rpm for 5 min. pipette out 3 ml of above clear solution and transfer it to 25 ml volumetric flask and make up the volume with mobile phase.

**Optimized Chromatographic conditions**


**RESULTS AND DISCUSSIONS**

A simple, precise and accurate HPLC method was developed for the estimation of Donepezil in uncoated formulations, consisting of methanol: phosphate buffer system (50:50% v/v). The chromatographic condition was set at a low rate of 1 ml/min with the UV detector at 230 nm. The above method was optimized with a view to develop an assay method for Donepezil.

Several mobile phase compositions were tried to resolve the peaks of Donepezil. The optimum mobile phase containing Methanol, KH2PO4 buffer (60:40 % v/v) was selected because it was found ideal to resolve the analyte peaks of the drugs. Quantification was achieved with UV detections at 230 nm based on peak area and absorbance. As per USP requirements system suitability studies were carried out in freshly prepared standard solution of Donepezil. Various parameters obtained with 20 µl of injection volume are summarized in the Table No.1.

The system is suitable for tailing factor, theoretical plate, resolution (Fig No.1&2). The method was specific for the drugs (fig No.6 &7). The data obtained from the precision experiments. The RSD value for precision was indication that the method was efficiently precise.

It is evident that the responses for Donepezil are strictly linear (fig No.5) in the studied concentration range, which is evident from the RSD values, slope, intercept and correlation. The method worked well in the range from 100- 500 µg/ml which suggests full capacity for the quantification of Donepezil. The regression coefficient was found to be 0.999. Percentage recovery was calculated from 80% to 120% by injecting in HPLC. The excellent recovery was made at each added concentration. There is allowable variation in flow rate, wave length which indicates that method is robust enough. The LOD for Donepezil was found to be 7.5 µg/ml. The LOQ for Donepezil was found to be 2.27 µg/ml.

The chromatogram of sample showed a single peak at the retention time of Donepezil indicating that there is no interference of changing the persons for injecting the sample into the instrument.
Fig. 4: Donepezil HCL sample R.T is 4.287

Fig. 5: linearity graph

Fig. 6: HPLC-UV chromatograms obtained for blank

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Limit</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>System suitability (%RSD of tailing factor)</td>
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<td>0.13</td>
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<tr>
<td>2.</td>
<td>Specificity</td>
<td>No interferences</td>
<td>Specific</td>
</tr>
<tr>
<td>3.</td>
<td>Precision: (a) System precision (b) Method</td>
<td>RSD NMT 2.0% RSD NMT 2.0%</td>
<td>0.25% 0.34%</td>
</tr>
<tr>
<td>4</td>
<td>Linearity</td>
<td>Correlation coefficient NLT 0.999</td>
<td>0.999</td>
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<tr>
<td>5</td>
<td>Accuracy</td>
<td>%Recovery range 98-102 %</td>
<td>99.9%</td>
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<tr>
<td>6</td>
<td>Robustness</td>
<td>RSD NMT 2%</td>
<td>Robust</td>
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<tr>
<td>7</td>
<td>LOD</td>
<td>S:N Ratio should be more than 3:1</td>
<td>7.5</td>
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<tr>
<td>8</td>
<td>LOQ</td>
<td>S:N ratio should be more than 10:1</td>
<td>2.27</td>
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**Table 1: Validation and system suitability parameters**

**CONCLUSION**

The reliability and suitability of the method could be seen from recovery studies. Further there is no interference due to excipients. System suitability parameters were calculated which includes efficiency, resolution and tailing factor. Precision of the methods were studied by making repeated injections of the samples and
system precision values were determined. The method was validated for linearity, accuracy, precision, robustness. The method is simple, specific & easy to perform and requires little time to analyse the samples. Low limit of quantification and limit of detection makes this method suitable for quality control. This method enables determination of Donepezil because of good separation and resolution of the chromatographic peaks. The method was found to be accurate, precise and robust. Hence it was concluded that the RP-HPLC method developed was very much suitable for routine analysis. Donepezil in tablet formulations and future planning using this method for the estimation Donepezil in clinical trials can be tried successfully.

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REFERENCES