

RP-HPLC METHOD DEVELOPEMENT AND VALIDATION OF DONEPEZIL HYDROCHLORIDE

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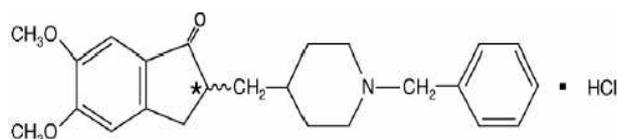
ABSTRACT

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, dimethoxy-2-[1-(phenylmethyl) - piperidynyl] -H Indane-1-one HCl ² (Fig No: 1) Donepezil is a specific noncompetitive ³ reversible inhibitor of acetylcholinesterase (AChE) ^{3, 4, 5}, and appears to exert its therapeutic effect by enhancing cholinergic function ^{4, 5} (As the dementia progresses, fewer cholinergic neurons are thought to remain functionally intact, and the effects of Donepezil may be lessened ^{4, 5} Donepezil exhibits a relatively high degree of selectivity for neuronal AChE ^{3, 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 AChE in the brain but causes little inhibition of AChE in smooth, striated, or cardiac muscle ^{3, 7} Donepezil inhibition of AChE in red blood cells corresponds closely to its effect at synapses in the central nervous system (CNS) ^{3, 6, and 8}. AChE inhibition in red blood cells has been used as an indicator of the clinical effectiveness of Donepezil in Alzheimer's disease patients ³.



±2, 3-dihydro 5, 6, dimethoxy-2-[1-(phenylmethyl) - piperidynyl] - 1H Indane-1-one HCl (2)

Fig. 1: 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) ⁹⁻¹¹, fluorescence (FL) ^{12, 13} or mass spectrometric (MS) detector ^{14, 15}. Solvent extraction spectrophotometry ¹⁶ spectrofluorimetry ¹⁷ and colorimeter ¹⁸ 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. Extraction¹⁶ 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

Equipment : HPLC Pump: (LC – 10 AT Vp series) Detector: UV – Visible Model SPD 10 Injector : Rheodyne Column : C₁₈ Elico PH – Meter Elico – SL 164 – Double beam UV - Visible spectrophotometer Gelman Science Vacuum Pump A & D – Digital Balance and Sonicator

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: (λ_{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 KH₂PO₄ 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 0.7gm of ground tablet powder (equivalent to 10mg of Donepezil hydrochloride) transfer it in 100 ml of volumetric flask, and add 100ml 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 rotary 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 25ml volumetric flask and make up the volume with mobile phase.

Optimized Chromatographic conditions

The chromatographic system consisted of a Column: C₁₈ (4.6 X 250 mm 3.5µm). Column temperature: 250°C, Flow rate: 1ml/min. Injection volume: 20 µl. Wavelength: 230 nm. Run time: 15 min. Diluents: Mobile phase. Mobile phase composition: Methanol: buffer (60:40%v/v). Pump: Phenomenex. Injector: Rheodyne. Stationary phase: Phenomenex C₁₈ 4.6 X 150 mm, 3.5µm. Operating temperature: Room temperature

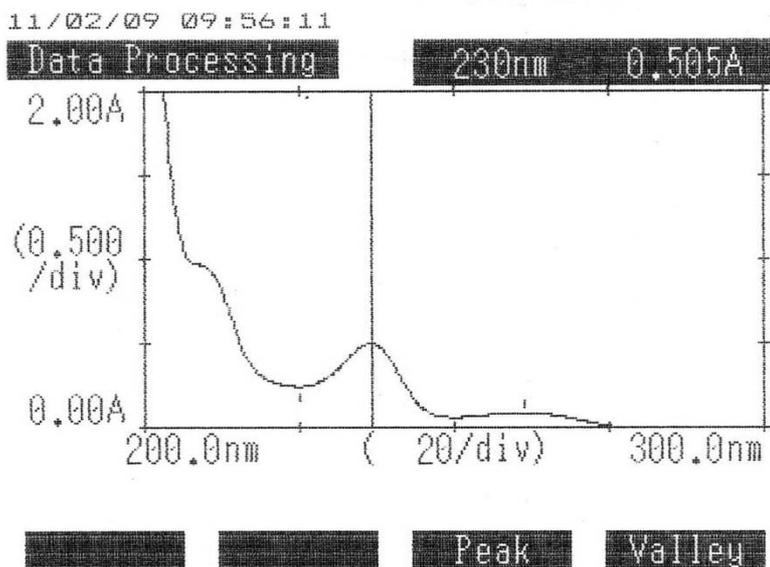


Fig. 2: UV Spectrum for Donepezil hydrochloride

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 20µl 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

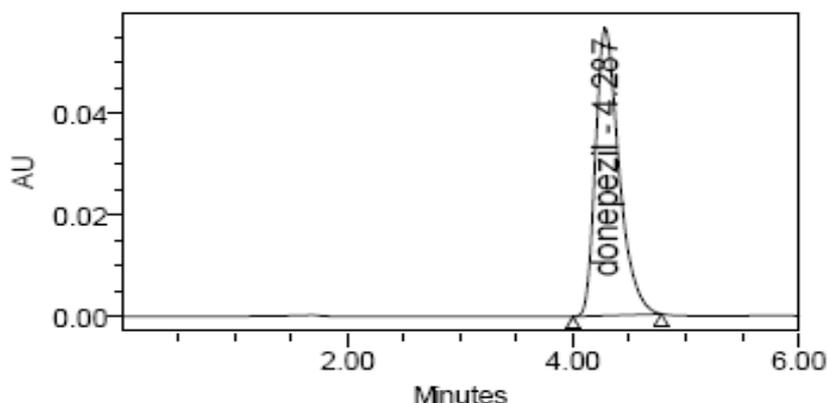


Fig. 3: Donepezil HCL STD R.T is 4.287

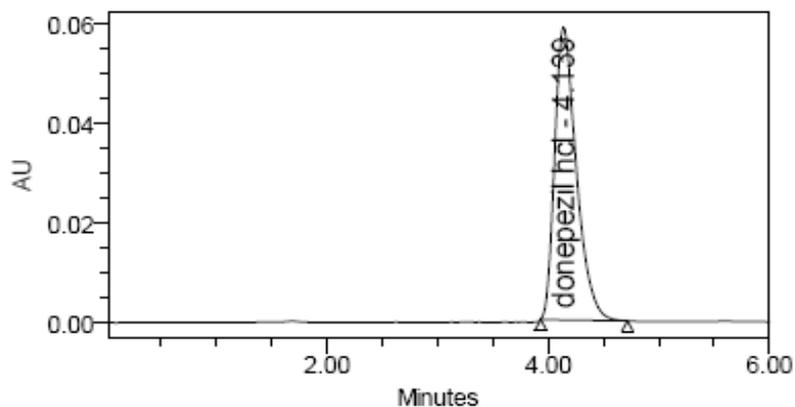


Fig. 4: Donepezil HCL sample R.T is 4.287

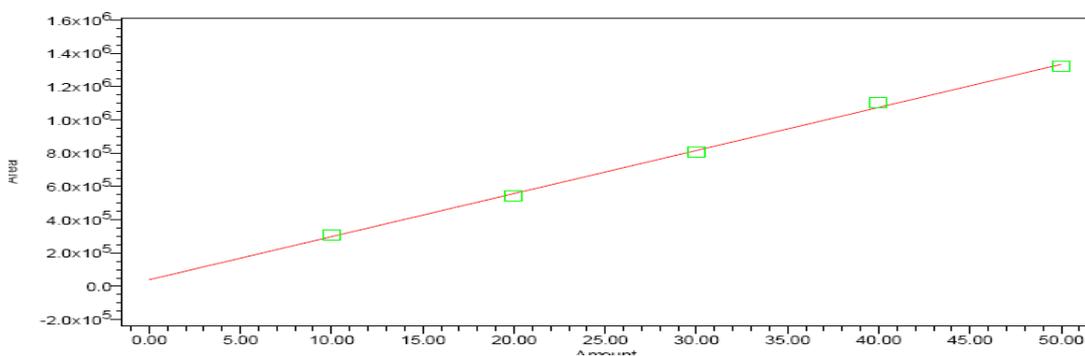


Fig. 5: linearity graph

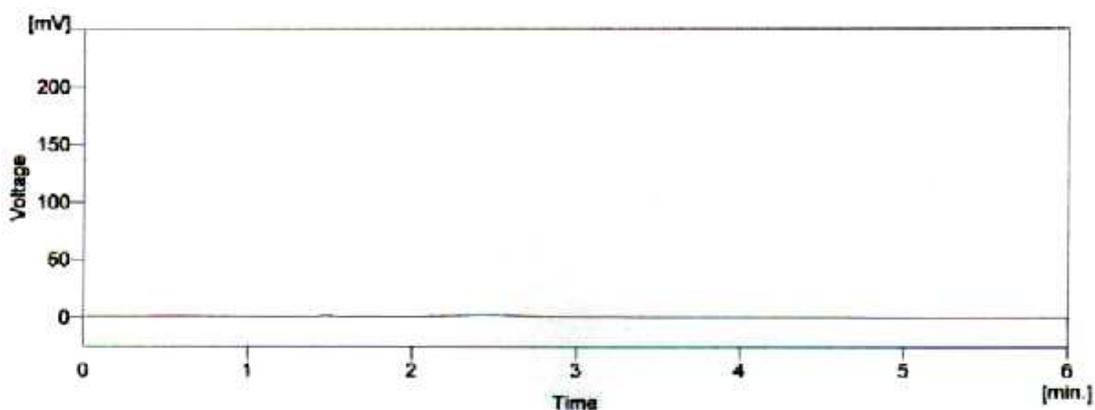


Fig. 6: HPLC-UV chromatograms obtained for blank

Table 1: Validation and system suitability parameters

S. No	Parameters	Limit	Observation
1	System suitability (%RSD of tailing factor)	Suitable	0.13
2	Specificity	No interferences	Specific
3	Precision: (a) System precision (b) Method precision	RSD NMT 2.0% RSD NMT 2.0%	0.25% 0.34%
4	Linearity	Correlation coefficient NLT 0.999	0.999
5	Accuracy	%Recovery range 98-102 %	99.9%
6	Robustness	RSD NMT 2%	Robust
7	LOD	S:N Ratio should be more than 3:1	7.5
8	LOQ	S:N ratio should be more than 10:1	2.27

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

- Maryyadele J O'Neil Editors. The Merck index 14th ed., Merck &co. Inc. Whitehouse station (NJ), 2006, pp 578
- Randy Hendrickson, editor. Remington—the science and practice of pharmacy 21st ed. Vol II, Lippincott-williams & Wilkins 2006, pp 1396
- Rogers SL, Friedhoff LT. The efficacy and safety of donepezil in patients with Alzheimer's disease: results of a US multicentre, randomized, double-blind, placebo-controlled trial. Donepezil Study Group. *Dementia* 1996; 7: 293-303.
- Aricept package insert (Eisai—US), donepezil (systemic) Rev 4/98, Rec 4/1/99.
- Aricept product monograph (Eisai—Canada), Donepezil (Aricept) for Alzheimer's disease Rev 8/8/97, Rec 10/29/97.
- Dickinson BD, editor. UHC drug monograph: Donepezil. UHC (University HealthSystem Consortium) Services Corporation; 1996. p. 1-14.
- Rogers SL, Farlow MR, Doody RS, et al. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Donepezil Study Group. *Neurology* 1998 Jan; 50: 136-45.
- Rogers SL, Friedhoff LT. Long-term efficacy and safety of donepezil in the treatment of Alzheimer's disease: an interim analysis of the results of a US multicentre open label extension study. *Eur Neuropsychopharmacol* 1998; 8(1): 67-75.
- Radwan M A, Abdine H H, Al-Quadeb B T, Aboul-Enein H Y, Nakashima K.J. Stereoselective HPLC assay of donepezil enantiomers with UV detection and its application to pharmacokinetics in rats. *J. Chromatogr B* 2006; 830: 114-119.
- Horacio Pappa, Romina Farru Paula Otaño Vilanova, Marcelo Palacios, María Teresa Pizzorno. A method to determine donepezil hydrochloride in tablets. *Journal of Pharmaceutical & Biomedical Analysis* 2002; 27(1-2): 177-182.
- Useni Reddy Mallu, K. Hussain Reddy, Varaprasad Bobbarala, Somasekhar Penumajji. RP- HPLC method development for the determination of assay of Donepezil hydrochloride. *International Journal of Chemical and Analytical Science* 2010; 1(2): 35-36.
- Haginaka J, Seyama C. Rapid and sensitive determination of donepezil in human plasma by liquid chromatography. *J Chromatogr* 1992; 577: 95- 102.
- Nakashima K, Itoh K, Kono M, Nakashima M N, Wada M. Determination of donepezil hydrochloride in human and rat plasma, blood and brain microdialysates by HPLC with a short C30 column. *J Pharm Biomed Anal* 2006; 41: 201-206.
- Lu Y, Wen H, Li W, Chi Y & Zhang Z. Determination of donepezil hydrochloride in plasma by liquid chromatography-mass spectrometry. *J Chromatogr Sci* 2004; 42: 234-237.
- Hao X Y, Ding L, Li L M, Bian X J, Zhang S Q. Rapid and sensitive determination of donepezil in human plasma. *Yao Xue Xue Bao* 2003; 38: 203-206.
- S Pillai & I Singhvi. Application of orange dye for quantitation of donepezil hydrochloride from tablet formulation. *Indian J Phar Sci* 2006; 68(5): 682-684.
- Samah Sayed Abbas, Yasmin Mohamed Fayed & Laila El-Sayed Abdel Fattah. Stability indicating methods for determination of donepezil hydrochloride according to ICH guidelines. *Chem Pharm Bull* 2006; 54(10): 1447-1450.
- J N Sangshetti, P R Mahaparale, S Paramane, D B Shinde. Spectrophotometric estimation of donepezil hydrochloride in bulk and tablet formulation. *Trends in Applied Sci Res* 2008; 3(1): 109- 112.