

## ESTIMATION OF DONEPEZIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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### ABSTRACT

A rapid and precise reverse phase High performance liquid chromatographic method has been developed for the estimation of Donepezil in its pure form as well as in pharmaceutical dosage forms. Chromatography was carried out on a C<sub>18</sub> Column (150 x 4.6mm) using a mixture of Di Hydrogen Orthophosphate Buffer (pH 3.5±0.05), Acetonitrile and methanol in a ratio of (15:20:65 v/v) as the mobile phase at a flow rate of 1.0 mL/min the detection was done at 225nm. The retention time of the drug was 3.6 min. The method produced linear responses in the concentration range of 80-160 µg/ml of Donepezil. The method was found to be reproducible for analysis of the drug in tablet dosage forms.

**KEY WORDS:** Donepezil, Estimation, RP-HPLC.

### 1. INTRODUCTION

Donepezil, (+ 2, 3. Dihydro 5,6 dimethoxy 2-[1-(phenyl/methyl) – piperidynyl]. 1-H Indane – 1 – one Hcl. It is Acetyl cholinesterase Inhibitor and it prevents the break down of acetylcholine in the brain. A literature survey revealed that only a few HPLC (Useni Reddy,2010; Maryam Hussein; Tushar and Patel,2009; Rajesh Sharma,2010) methods are available for the estimation of Donepezil. The authors now propose a new validated, sensitive and reproducible HPLC method for the determination of Donepezil and the dosage forms was also observed.

### 2. EXPERIMENTAL

**Chromatographic conditions :** A prominence isocratic HPLC system (waters) C<sub>18</sub> ODS column (150x4.6mm, 5µ) with a UV-visible detector was employed in the study. A 20µL Hamiton injection syringe was used for sample injection. HPLC grade Di Hydrogen Orthophosphate Buffer, Acetonitrile and methanol and were used for the preparing the mobile phase. A freshly prepared (15:20:65v/v) mixture of buffer, Acetonitrile and methanol was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.0mL/min. the column temperature was maintained at room temperature, the detection of the drug was carried out at 225nm.

**Preparation of Standard Solutions:** Weigh accurately Donepezil working standard equivalent to about 20 mg of Donepezil in to 20mL of volumetric flask, add 15mL of diluents and sonicate to dissolve for about 10min, further make up the volume with diluents. And dilute 1mL to 10mL with Acetonitrile and methanol. Subsequent dilutions of this solution ranging from 80-160µg/mL were made in 10mL volumetric flask to each dilution. Each dilution was injected 5 times in to the column (20mL) and the corresponding chromatographs were obtained, from these chromatograms the ratio of the area under the peak of the drug was calculated. The regression of the drug concentrations over the ratios was computed. This regression equation obtained was used to estimate the amount of Donepezil in pharmaceutical dosage forms.

Solutions containing 80-160µg/mL of Donepezil were subjected to the proposed HPLC analysis to check the inter day and intra day variation of the method by adding known amounts of Donepezil to the pre-analyzed samples and then analyzing samples and then analyzing them by the proposed method.

**Estimation of Donepezil in tablets:** Two commercial samples of the tablets containing the drug were chosen for testing the suitability of the proposed method to estimate Donepezil in tablets. For this, Weigh accurately quantity of the powdered tablets equivalent to about 10mg of Donepezil in to 100mL volumetric flask, add about 60mL of diluents, Sonicate for about 30min and dilute to 100ml with Acetonitrile and methanol. Filter through 0.45 micron filter. The



contents of the flasks were made up to the volume with the mobile phase and mixed well. Twenty micro liters of each of these solutions was then injected five times in to the column. The mean peak area ratios of the drug to the five such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

### 3.RESULTS AND DISCUSSION

The aim of this study was to develop a rapid and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Donepezil in its pure form as well as in pharmaceutical dosage forms. Chromatography was carried out on a  $C_{18}$  column (150 x 4.6mm) using a mixture of Di Hydrogen Orthophosphate Buffer (pH 3.5±0.05), Acetonitrile and methanol in a ratio of (15:20:65(v/v)) as the mobile phase at a flow rate of 1.0mL/min the detection was done at 225nm. The retention time of the drug was 3.6 min. The method produced linear responses in the concentration range of 80-160 µg/ml of Donepezil. The method was found to be reproducible for analysis of the drug in tablet dosage forms. The chromatogram is shown in Fig.1.

Each of the samples was injected 5 times and the same retention time was observed in all the cases. The ratio of the peak areas of Donepezil for the different concentrations taken up was calculated and the average value for 5 such determinations are shown in Table.1. The peak area of Donepezil was reproducible as indicated by low coefficient of variation. A good linear relationship ( $r=0.998$ ) was observed between the concentration of Donepezil and the respective ratios of peak areas in the concentration range of 80-160/mL of the drug. The linearity curve was constructed and its regression coefficient is  $Y=12961X+73841$ , when Donepezil solutions containing 80-160 µg/mL were analyzed by the proposed method for finding out the intra & inter day variations in the recoveries, A low coefficient of variation in the results was observed as shown in Table.2. This shows that the present HPLC method is highly precise. The amount of Donepezil obtained from the preanalyzed samples containing known amounts of added drug are shown in Table.3. About 99.93% of Donepezil could be recovered from the Pre-analyzed samples indicating high accuracy of the proposed method.

The drug content in the tablet was quantified by using the proposed analytical method. The tablets were found to contain an average of 99.93% of the labeled amount of the drug. The low coefficient of variation indicates the reproducibility of the assay of Donepezil in dosage forms. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of Donepezil in pharmaceutical dosage forms with in a short analysis time. The method was validated by the evaluation of the validated parameters.

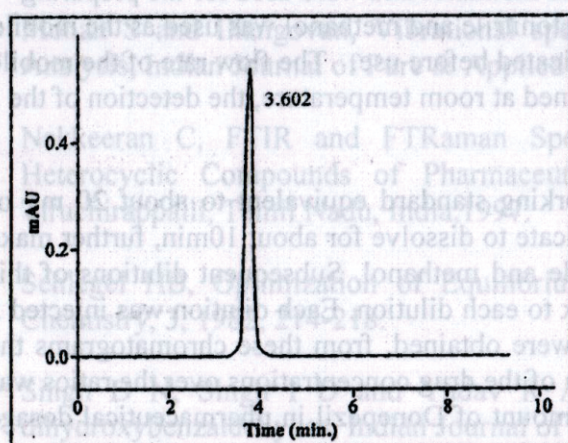


Fig: 1 A typical chromatogram of Donepezil

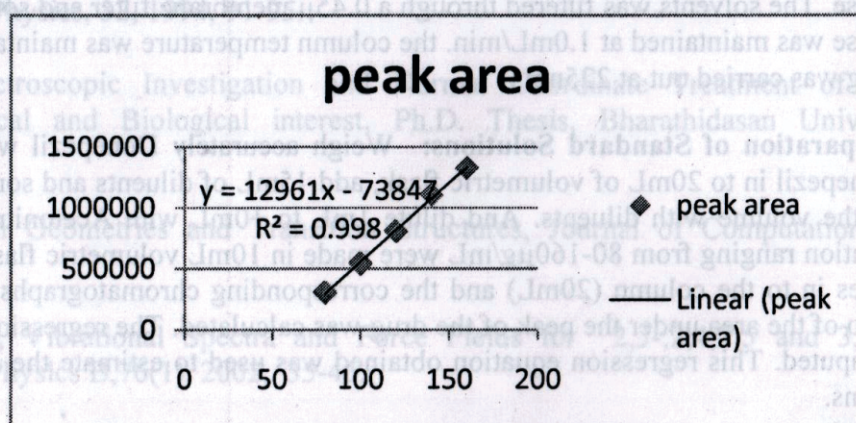


Fig:2: Linearity graph of Donepezil



S.No.	Concentration (mcg/ml)	Peak Area
1	80	307387
2	100	542671
3	120	807576
4	140	1103461
5	160	1323084

Table 1 - Calibration of the proposed method

Sample No.	Area	Amount%
1	817118	98.5
2	816535	99.2
3	816758	98.9
4	816022	100.0
5	812152	98.7
	Mean	98.9
	SD	0.63
	% RSD	0.64

Table 2 – Precision of proposed method

Concentration %	Amount (mg) added	Amount (mg) found	%Recovery	Mean
80	5	5.04	100.89	100.26
80	5	4.84	96.9	
80	5	5.15	103	
120	10	9.80	98.05	98.05
120	10	9.80	98.05	
120	10	9.80	98.05	
160	15	15.23	101.5	101.5
160	15	15.23	101.5	
160	15	15.23	101.5	

Table 3: Recovery data of Donepezil

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