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## Development and Validation for Estimation of Donepezil HCl from Bulk and Marketed Dosage Forms by Using RP-HPLC

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**Keywords:** RP-HPLC, Validation, Donepezil HCL, Aricept

### ABSTRACT

A simple, rapid, precise and accurate assay and validated RP-HPLC method was invented to quantify donepezil hydrochloride in drug solution and orally disintegrating tablet. An analytical column with C18 stationary phase (250 X 4.0mm.d.) bonded onto 5 $\mu$ m silica gel was used for chromatographic separation. Separation was achieved using spectrophotometric. Isocratic mode of separation with mobile phase methanol: 0.02m phosphate buffer: Triethylamine (50:40:10)% v/v were used. The flow rate was 1ml/min. Linearity for Donepezil HCL were in the range of 40mcg/ml - 120mcg/ml. Amount found of Donepezil HCL in Aricept 5mg, Aricept10mg, Donepezil 5mg tablets were 5.0072mg/tab, 10.01mg/tab and 5.01mg/tab respectively. Percentage recovery obtained was 100.53%, 100.24% and 100.34%. The proposed method is accurate, precise, selective and rapid for the estimation of Donepezil HCL in tablet dosage.

## INTRODUCTION

Donepezil is a cholinesterase inhibitor. It works by increasing the amount of a certain substance (acetylcholine) in the brain, which may help reduce the symptoms of dementia in patients with Alzheimer disease. It is developed by Eisai and Pfizer and is sold as a generic by multiple suppliers [1]. Donepezil acts as a centrally acting reversible acetylcholinesterase inhibitor, it is available in a white powder with crystalline structure. It can be dissolved in methanol, water and glacial acetic acid slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. It has recently been approved for marketing in the USA, Canada and several EU member states, including the UK, for the symptomatic treatment of mild to moderate Alzheimer's disease [2]. Donepezil hydrochloride is commonly referred to in the pharmacological literature as E2020. It has an empirical formula of  $C_{24}H_{29}NO_3HCl$  and a molecular weight of 415.96 with melting point of 218-220°C. It is not yet official in any pharmacopeia [3]. The literature survey

Revealed that a number of methods being reported for the estimation of donepezil hydrochloride in biological samples. There is some reported method for the estimation of the above drug in tablets by Reverse Phase HPLC method. The objective of the study was to develop an HPLC method with short retention time, high sensitivity and applicable for quantification of donepezil hydrochloride in drug solution. The method was then applied to assay donepezil hydrochloride.

## MATERIALS AND METHODS

### Materials

Donepezil hydrochloride was obtained from Cipla Bangalore as a gift sample [Batch No-F120234]

### Instrumentation

Shimadzu HPLC model SPD-M20A was used for method development and validation. PDA detector is used. It is a multichannel detector contains an ideal sensor for an entire spectrum in a UV/VIS dispersive spectrophotometer. These are useful in both research and quality assurance laboratories and provides users most advanced level of sensitivity. Eclipse plus  $C_{18}$  columns are designed for superior peak shape with basic compounds and deliver high

efficiency and excellent peak shape with all sample types. Eclipse plus C<sub>18</sub> is especially useful for the separation of acidic, basic, and other highly polar compounds by reverse-phase liquid chromatography (250 × 4.6 mm, particle size is 5 μm). The mobile phase consisted of a mixture of A, B and C which was filtered through a membrane filter 4.5 μm. The solvents were degassed before running at a flow rate of 1 ml/min. The column temperature was ambient at 30 °C. The 20 μl volume of sample was injected and peaks were detected at 158 nm.

#### **Preparation of 0.02M Phosphate Buffer**

Mix pH 8 of 50ml has 0.2M KH<sub>2</sub>PO<sub>4</sub> and 46.8ml of 0.02M sodium hydroxide and makeup up to 500ml with distilled water

#### **Preparation of 0.2M Potassium Hydrogen Phosphate**

27.218 gm. of potassium hydrogen phosphate was weighed and dissolved in little amount of distilled water. Then the contents were shaken thoroughly and makeup up to 1000ml with distilled water. Then adjust to pH8 with phosphoric acid.

#### **Preparation of 0.2M Sodium Hydroxide**

8 gram of sodium hydroxide was accurately weighed and dissolved in little amount of distilled water. Then the contents were shaken thoroughly and makeup up to 1000ml with distilled water.

#### **Preparation of Mobile Phase**

Mix 600ml of methanol, 400ml of 0.02M phosphate buffer and 5ml of triethylamine. Then adjust to pH 7.5 with phosphoric acid to form 1000ml of mobile phase. This was finally filtered through membrane filter of micron 0.45mm, degassed and this is used as the mobile phase.

#### **Preparation of Standard Stock Solution**

Weight 50mg of standard drug of Donepezil HCL and transferred to a 50 ml standard volumetric flask, dilute with little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 50ml with the same mobile phase. From the above solution, take 2ml and transferred to 50ml standard volumetric flask and dilute with

little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 50ml with the same mobile phase.

### Preparation of Sample Solution

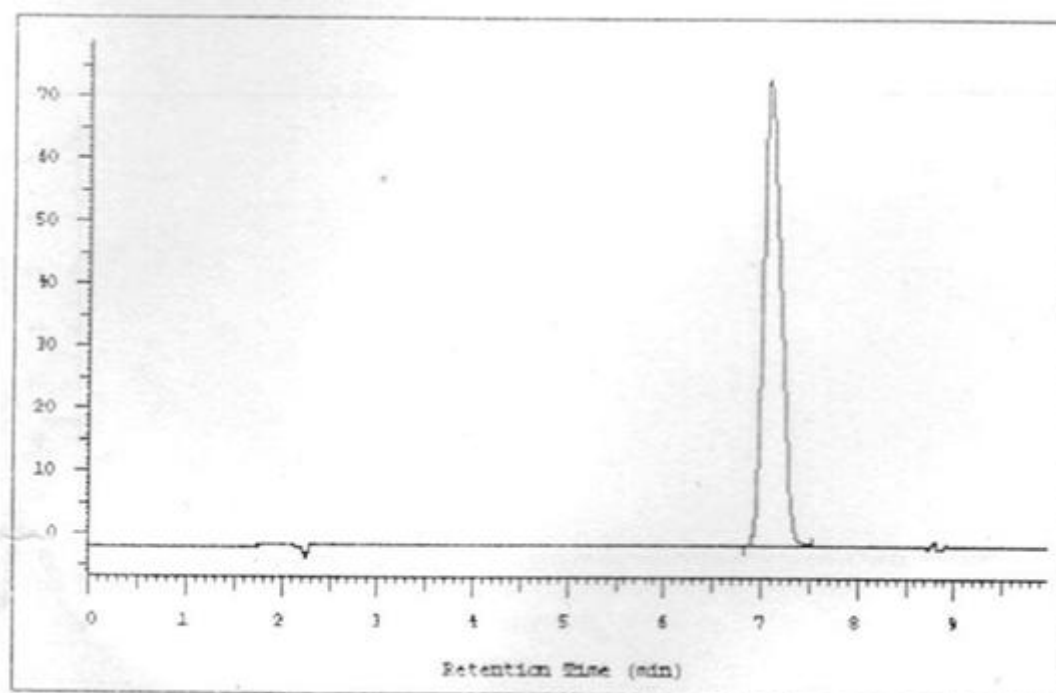
Ten tablets were weighed and crushed to obtain a fine powder. Powder equivalent to 5 mg of Donepezil HCL was weighed accurately and transferred to a 50ml standard volumetric flask, dilute with little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 50ml with the same mobile phase to give the concentration of 100mcg/ml of Donepezil HCL.

## RESULTS AND DISCUSSION

### Assay

20  $\mu$ l of standard stock solution and sample solution were injected into an injector of liquid chromatography. From the peak area of Donepezil HCL, the amount of drug in sample was computed. A typical chromatogram of Donepezil HCL (Fig. 1)

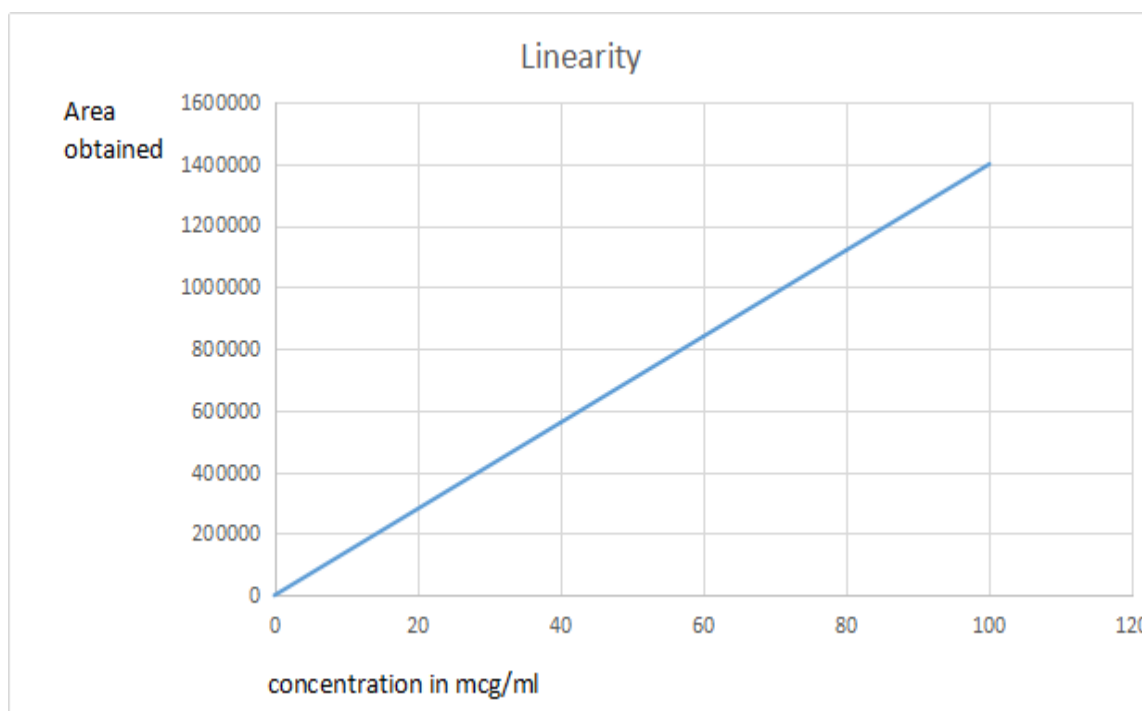
Fig 1



In the proposed method the content of Donepezil HCL in Aricept 5mg, Aricept 10mg and Donepezil 5 mg were found to be 5.0072mg/tab, 10.01 mg/tab and 5.016 mg/tab respectively. The results obtained by the proposed method were close to the label claim of the three drugs indicating that the method is precise and accurate.

### Linearity Study

Into a series of varying amount of standard Donepezil HCL solution was taken and made up to different concentrations of 40mcg/ml, 60 mcg/ml, 80mcg/ml, 100 mcg/ml and 120 mcg/ml. 20 $\mu$ l was injected from each flask. Peak area responses of the solutions were recorded at 158nm. The plots of peak area versus the respective concentrations of Donepezil HCL were found to be linear in the range of 40 mcg/ml - 120mcg/ml Fig.2. The correlation coefficient of Donepezil HCL was found to be 0.9970.



### Recovery Study

Recovery of the method was observed by the results from 3 placebo preparations accurately spiked with different concentration of the active ingredient (Donepezil HCL). The results are reported since there is no significant difference between the theoretical and actual amounts, the method is shown to be accurate and selective. A relative standard deviation of less than 2% was obtained which proves accuracy (Table. 1).

**Table 1: Donepezil Hydrochloride Tablets**

Concentration(mcg/ml)	Result obtained(mg)	% Recovery
20	5.0269	100.40
40	5.0124	100.24
50	5.0174	100.34
Mean% recovery	-	100.32
Standard deviation	-	0.08
%RSD	-	0.0008

**System Suitability**

System suitability tests were carried out on freshly prepared standard stock solution of Donepezil HCL and the parameters obtained with 20µl injection volume are shown in (Table.2).

**Table 2: System Suitability**

Peak No.	Area(mvs)	Retention time(sec.)
1	1119930	7.06
2	1119942	7.05
3	1119854	7.05
4	1119944	7.03
5	1119932	7.05
6	1119958	7.06
Average	1119926.667	
% RSD of peak area	0.003301937	

**Instrumental Precision**

It is established by repetitive injection of the same standard solution ten times, followed by the averaging of the peak area and determination of the %RSD of all the injections. The % RSD value obtained was 0.00330, which indicates precision of the method.

### **Intra-Assay Precision**

It is established by multiple measurements of the same sample (different preparations) by the same analyst under the same conditions. The %RSD value obtained was 0.1034, which indicates precision of the method.

### **Intermediate Precision**

#### Analyst to Analyst Variability

The % RSD value obtained for Analyst1, Analyst 2 was 0.05765 and 0.03150 respectively.

#### System to System Variability

The %RSD value obtained for system1, system 2 was 0.012543 and 0.03398 respectively. The %RSD value was obtained, which indicates precision of the method.

### **CONCLUSION**

The proposed RP-HPLC method is accurate, precise, rapid, and selective for the determination of the Donepezil HCL in tablets dosage. Hence it can be conveniently adopted for the routine quality control analysis.

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