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Development and validation of RP- HPLC method for estimation of Donepezil HCl from bulk and marketed dosage forms

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ABSTRACT

A simple, fast and precise Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method developed for the determination of Donepezil Hcl tablets, C_{18} column 250mm x 4.6mm(l x d) in reverse phase isocratic mode of separation with mobile phase methanol : 0.02m phosphate buffer : Triethylamine (60:40:0.5)% v/v were used. The flow rate was 1ml/min. Linearity for Donepezil Hcl were in the range of 50mcg/ml - 150mcg/ml. Amount found of Donepezil Hcl in Aricept 5mg, Aricept10mg, Dopezil 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.

Key words: RP-HPLC, Vlidation, Donepezil Hcl, Aricept.

INTRODUCTION

Donepezil is an oral medication used to treat Alzheimer's disease. Chemically the drug is 2, 3-dihydro-5, 6-dimethoxy-2-[(1-(phenyl methyl)-4-piperidinyl) methyl]-1*H*-inden-1-one hydrochloride. Several methods such as HPLC [1], LC/MS/MS [2], HPLC/MS [3-7]. Donepezil has been tested in other cognitive disorders including Lewy body dementia [8] and vascular

dementia [9] but it is not currently approved for these indications. Donepezil has been found to improve sleep apnea in Alzheimer's patients [10]. The studies found that speech of austistic children who were mild to moderately affected appeared to improve from the use of Donepezil [11, 12] medication [13].

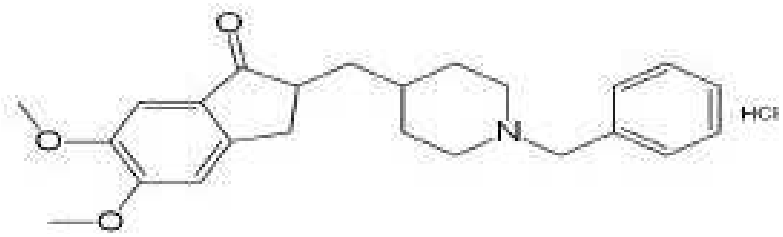

Fig 1: Structure of Donepezil Hydrochloride

EXPERIMENTAL SECTION

High performance liquid chromatography, Pump isocratic LC20AT YP equipped with rheodyne injection volume 20µl, UV detector UV-HRT. Reference standard of Donepezil Hcl. Aricept 5mg, Aricept 10mg,Dopezil 5mg tablets were procured from market. Methanol HPLC grade, Water of HPLC grade, 0.2M phosphate buffer HPLC grade. Stationary phase C_{18}, 5µ, Column 250mm x 4.6mm (l x d) were used.

Preparation of 0.02M Phosphate Buffer

Mix pH 8 of 50ml have 0.2M KH_2PO_4 and 46.8ml of 0.02M sodium hydroxide and make 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 make 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 make 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 pH7.5 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 31.2mg of standard drug of Donepezil Hcl and transferred to a 25 ml standard volumetric flask, dilute with little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 25ml with the same mobile phase. From the above solution, take 2ml and transferred to 25ml standard volumetric flask and dilute with little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 25ml with the same mobile phase.

Preparation of Sample Solution

Ten tablets were weighed and crushed to obtain a fine powder. Powder equivalent to 2.5 mg of Donepezil Hcl was weighed accurately and transferred to a 25ml standard volumetric flask, dilute with little amount of mobile phase and the contents were shaken thoroughly and finally make up the volume to 25ml with the same mobile phase to give the concentration of 100mcg/ml of Donepezil Hcl.

RESULTS AND DISCUSSION

Assay

20 µl of standard stock solution and sample solution were injected in to 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. 2)

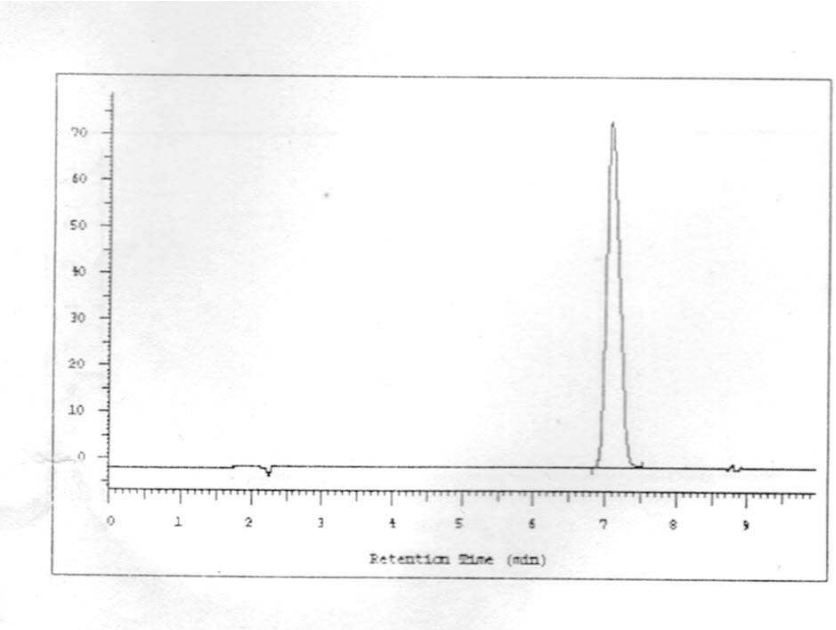

Fig 2: A Typical Chromatogram of Donepezil Hcl

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

In to a series of varying amount of standard Donepezil Hcl solution was taken and made up to different concentrations of 50mcg/ml, 75 mcg/ml, 100mcg/ml, 125 mcg/ml and 150 mcg/ml. 20µl was injected from each flask. Peak area responses of the solutions were recorded at 168nm. The plots of peak area versus the respective concentrations of Donepezil Hcl were found to be linear in the range of 50 mcg/ml - 150mcg/ml Fig.3. The correlation co-efficient of Donepezil Hcl was found to be 0.9970.

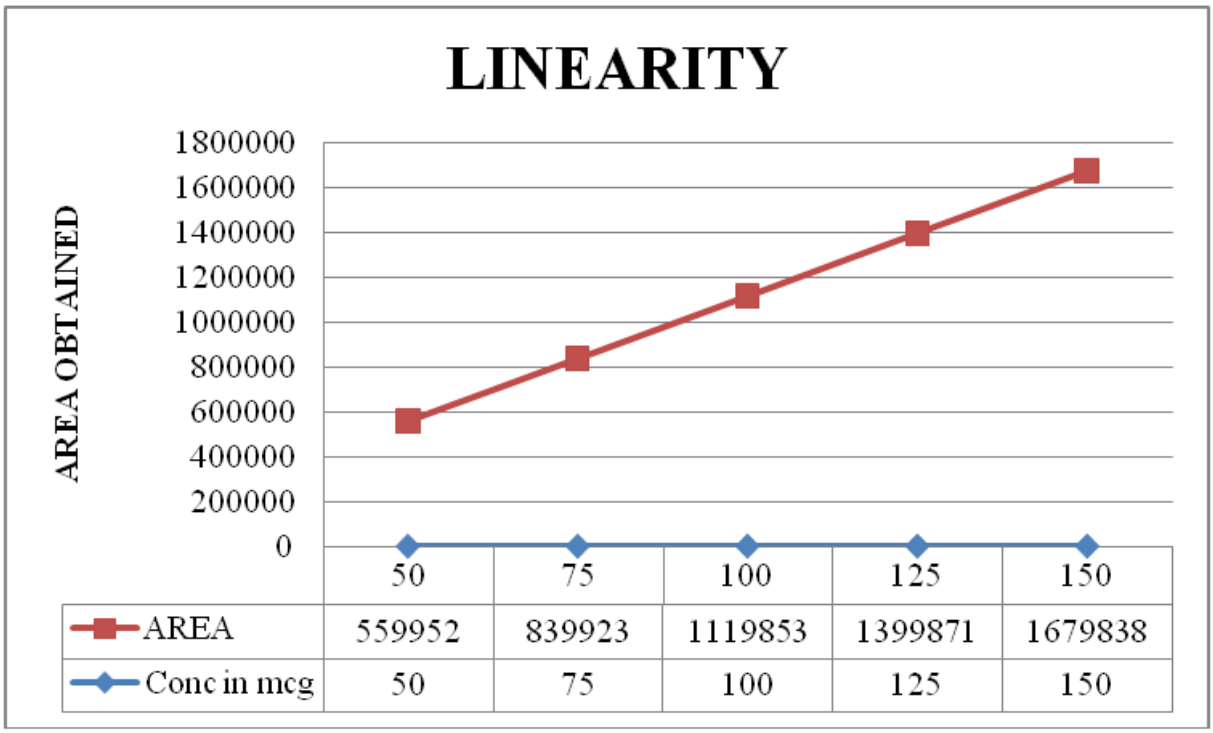

Fig 3: Linearity Graph of Donepezil Hcl

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	100.53
40	5.0124	100.24
50	5.0174	100.34
Mean% recovery	-	100.37
Standard deviaton	-	0.1473
%RSD	-	**0.1467**

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). High number of theoretical plates indicates efficient performance the column.

Table 2: System Suitability

Peak No.	Area(mvs)	Retention time(sec.)	No. of theoretical plates	Tailing factor
1	1119945	7.06	1523	1.11
2	1119948	7.06	1529	1.12
3	1119884	7.05	1524	1.11
4	1119942	7.04	1529	1.10
5	1119937	7.04	1523	1.11
6	1119953	7.05	1524	1.11
Average	1119934.83	7.05	1525.33	1.11
% RSD of peak area	**0.002275**	-	-	-

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 0.0429, 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 0.2034, which indicates precision of the method.

Intermediate Precision

Analyst to Analyst Variability

The % RSD value obtained for Analyst1, Analyst 2 was 0.005765 and 0.01150 respectively.

System to System Variability

The %RSD value obtained for system1, system 2 was 0.011493 and 0.02289 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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REFERENCES

[1] K Nakashima; K Itoh; MN Nakashima; M Wada. *J-pharm-Biomed.*, **2006**, 41(1), 201-206.
[2] Z Xie; Q Liao; X Xu; M Yao; J Wan; D Liu. *Rapid commun mass Spectrum.*, **2006**, 20(21), 3193-8.

[3] YH Lu; HM Wen; W Li; YM Chi; ZX Zhang. *Acta pharmaceutica Sinica* (*Yao xue xue bao*)., **2003**, 38(3), 203-6.
[4] Vinay Saxena; Zahid Zaheer; Farooqui. *J.pharm.Edu.Res*., **2006,** 40(2), 100-105.
[5] R Gotti; V Cavrini; R Pomponio; V Andrisano. *J.pharm Biomed*., **2001**, 24(5-6), 863-70.
[6] K Matsui; Y Oda; H Ohe; S Tanaka; N Asakawa. *J chromatogr*., **1995,** 694(1), 209-18.
[7] A Golcu; SA Ozkan. *Pharmazie*., **2006,** 61(9), 760-5.
[8] CH Rojas-Fernandez. *Ann Pharmacother.*, **2001**, 35 (2), 202–205.
[9] R Malouf; J Birks. *Cochrane Database Syst Rev.*, **2004** (1), CD004395. doi: 10.1002/ 4651858.CD004395.pub2. PMID 14974068.
[10] W Moraes; D Poyares; L Sukys-Claudino; C Guilleminault; S Tufik. *Chest.*, **2008**, 133 (3), 677–83.
[11] Alzheimer's Drug Shows Promise as Treatment for Autism-Arehart-Treichel, *Psychiatric News* (pn.psychiatryonline .org), **2001**:11-16.
[12] http://pn.psychiatryonline.org/cgi/content/full/36/22/16-a.
[13] K Useni Reddy Mallu; Hussain Reddy; Varaprasad Bobbarala; Somasekhar Penumajji. *Int J of Ch and An Sci.*, **2010**, 1(2), 35-36.