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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE DETERMINATION OF DONEPEZIL USING RP-HPLC

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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, Xterra RP C_{18}, 250 x 4.6 mm, 5µ in reverse phase isocratic mode of separation with mobile phase Potassium dihydrogen orthophosphate Buffer and Acetonitrile (80:20)% v/v was used. The flow rate was 1ml/min. Detection was done at 230 nm. Linearity for Donepezil HCl were in the range of 25 µg/ml – 75 µg/ml. Percentage recovery obtained was 99.60%, 98.48 % and 98.1% for 50, 100 and 150 % respectively. The proposed method is accurate, precise, selective and rapid for the estimation of Donepezil HCl in tablet dosage.

Key words: *RP-HPLC, Donepezil Hcl, Potassium dihydrogen orthophosphate, Acetonitrile*.

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INTRODUCTION:

Donepezil hydrochloride is a cholinesterase inhibitor used in the treatment of Alzheimer's disease. Mainly it is available as its hydrochloride salt. Chemically it is 2-[(1-benzyl-4-piperidyl) methyl]-5, 6-dimethoxy-2, 3-dihydroinden-1-one hydrochloride. (Figure 1). Donepezil HCl is a White to off-white or slightly yellow crystalline powder with a molecular weight of 415.953. It is freely soluble in chloroform, dichloromethane and in methanol, soluble in water, sparingly soluble in ethanol, n-butanol and in acetonitrile and very slightly soluble in acetone [1-3]. As per the literature review, HPLC, HPTLC, LC-MS and few Spectrophotometric methods for the estimation of Donepezil in dosage forms or biological matrix were reported [4-17]. Most of the reported HPLC methods were cumbersome, time consuming and expensive. Hence the primary objective of the present work was to develop and validate a simple, economic, rapid and accurate Rp-HPLC method for the estimation of Donepezil HCl in its dosage form. [18, 19]

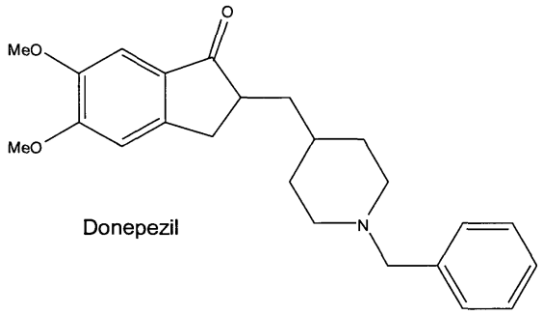

Fig 1: Chemical structure of Donepezil

MATERIALS AND METHODS:

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, tablets were procured from market. Acetonitrile HPLC grade was purchased from Merck, Mumbai. Water of HPLC grade and 0.2M phosphate buffer HPLC grade were purchased form SD Fine chem, Mumbai. Stationary phase Xterra RP $C_{18,}$ 250 x 4.6 mm, 5µ was used.

Preparation of Buffer:

Dissolve 2.72gm of potassium dihydrogen orthophosphate in 1000ml of Milli-Q water and filter through 0.45µm nylon membrane filter and degas.

Preparation of Mobile phase:

Prepare a degassed mixture of potassium dihydrogen orthophosphate and Acetonitrile in the ratio of 70:30% v/v.

Diluent: Buffer: Acetonitrile (50:50 v/v).

Preparation of Donepezil HCl Standard stock solution:

Weigh and transfer accurately about 50.0 mg of Donepezil HCl Working Standard into a 100 ml clean dry volumetric flask. Dissolve and dilute to volume with diluent. Transfer 5.0mL of above solution in to 50mL volumetric flask and dilute to volume with diluent and mix.

Preparation of Donepezil HCl Sample solution:

Transfer 10 tablets in to a 200mL volumetric flask, add about 20ml of buffer. Rotate on a rotary shaker until the tablets disintegrate completely, add about 100mL of Acetonitrile and sonicate for 20mins with occasional shaking (maintain the sonicator bath temperature between 20c 25).Dilute to volume with buffer and mix. Filter a portion of the solution through 0.45µm membrane filter and discard first few ml of the filtrate. Transfer 5.0mL of above solution in to 50mL volumetric flask and dilute to volume with diluent and mix.

Chromatographic Procedure:

Separately inject 10µl of diluent, placebo, standard preparation (5 times) and sample preparation into the chromatographic system. Record the chromatogram and measure the peak responses. Examine the placebo chromatogram for any extraneous peaks observed in the chromatogram of sample preparation.

RESULTS AND DISCUSSION:

Prepared standard and sample solutions as per test method and injected into the chromatographic system. Donepezil HCl at standard concentration level, and spiked to Donepezil HCl tablets 5 mg sample at specification level and injected into chromatographic system. Placebo solutions of donepezil HCl tablets USP 5 mg were prepared and injected into the chromatographic system as per methodology. The standard and sample solutions were comparable with respect to retention time. There are no interfering peaks observed due to placebo of Donepezil HCl tablets 10mg at the retention time of donepezil HCl Representative Chromatograms of blank, placebo, standard and sample solutions of Donepezil were shown in figure 2-5 respectively. The proposed method was validated as per the guidelines of ICH. [20]

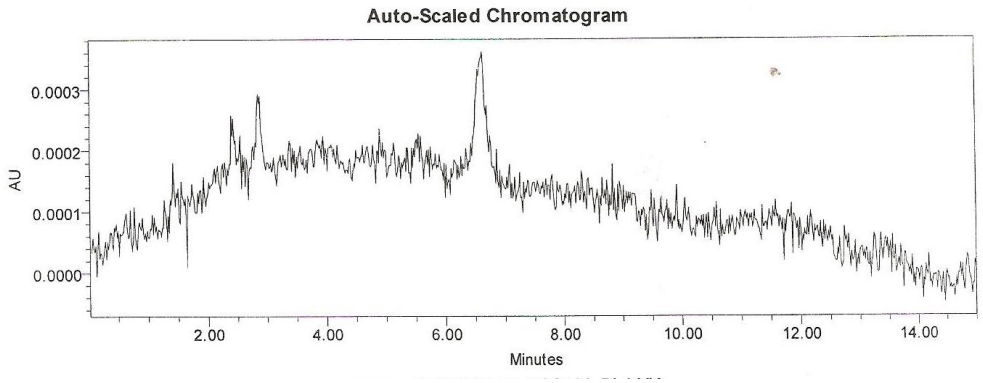

Fig 2: Blank Chromatogram

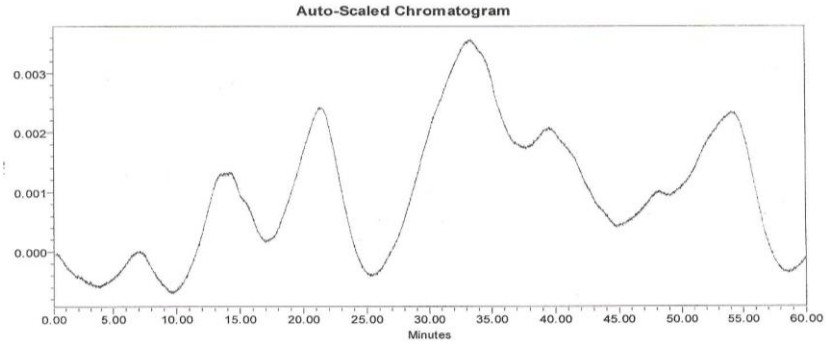

Fig 3: Placebo Chromatogram

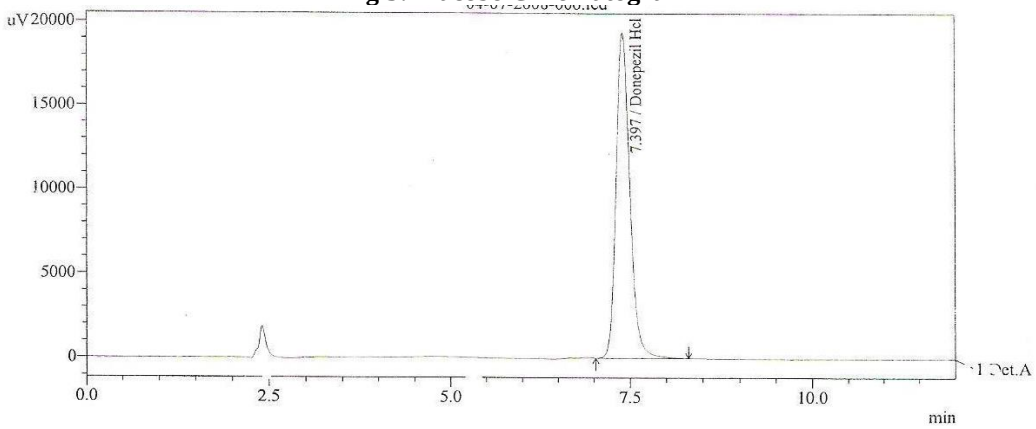

Fig 4: Standard chromatogram of Donepezil HCl

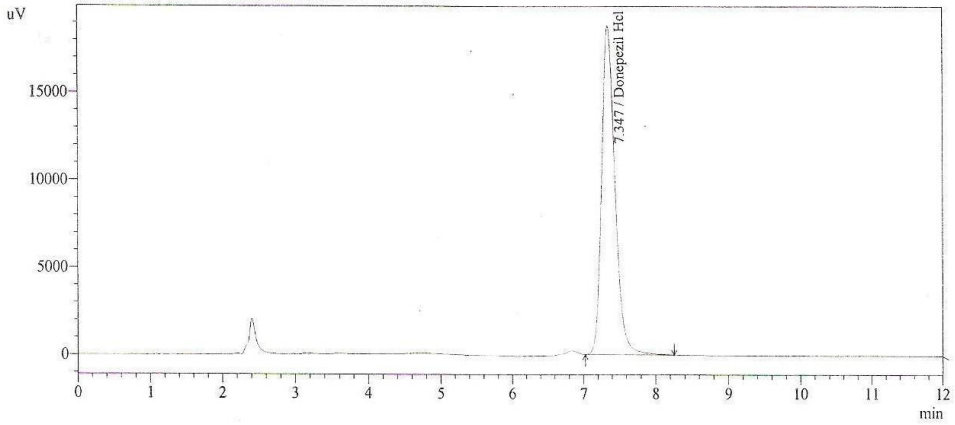

Fig 5: Sample chromatogram Donepezil HCl

Table 1: Results of system suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	7.077	5412895	31109	0.9
2	7.075	5380214	31351	0.9
3	7.077	5396184	30548	1.0
4	7.079	5348129	31986	1.1
5	7.075	5341765	31584	1.0
6	7.076	5471046	31245	0.9
Mean	7.077	5368087	31182	0.99
SD	0.029	47556.91	---	---
% RSD	0.38	0.88	---	---

System Suitability:
A Standard solution was prepared by using, Donepezil HCl working standard as per test method and was injected ten times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from ten replicate injections of Donepezil HCl retention times and peak areas. Table 1 shows the Results of system suitability.

Precision:
Prepared six sample preparations individually using a batch of tablets of Donepezil HCl (10mg) as per the test method and injected each solution. Table 2 shows the results of Precision.

Accuracy (Recovery):
A study of Accuracy was conducted. Drug recovery was performed in triplicate as per test method with equivalent amount of Donepezil HCl into each volumetric flask for each spike level to get the concentration of Donepezil HCl equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Donepezil HCl was calculated. Separately inject the blank, placebo, Donepezil HCl in to the chromatograph. Table 3 shows the results of % Recovery (Accuracy).

Linearity of Test Method:
A Series of solutions are prepared using Donepezil HCl working standard at concentration levels from 50% to 150% of target concentration (50%, 75%, 100%, 125% and 150%). Measure the peak area responses of solution at Level 1 and Level 6 for six times. Table 4 and figure 2 shows the linearity data and linearity curve respectively.

Table 2: Results of Precision

Tablet ID	Peak area	% Assay
1	1224800	99.6
2	1228138	97.8
3	1216863	100.3
4	1221207	99.8
5	1222479	98.9
6	1222697	99.2
Mean	1222697.33	99.26
SD	3750.47	0.86
%RSD	0.30	0.87

Table 3: Results of % Recovery (Accuracy)

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Mean % Recovery
50 % Sample	25.10	24.79	98.7	99.06
	25.05	24.46	97.6	
	25.05	24.53	97.9	
100 % Sample	50.20	49.45	98.2	98.46
	50.10	49.45	98.5	
	49.95	49.10	98.7	
150 % Sample	75.50	74.25	98.3	98.1
	75.50	74.10	98.1	
	74.95	73.40	97.9	

Table 4: Data of Linearity

Linearity Level	Average Area	Statistical analysis
L1-50%25	2684044	Y =106298.75x+0.1682
L2-60%	3220852	R^2 = 0.9813
L3-80%	4294470	Slope = 106298.75
L4-100%50	5368087	Y intercept = 0.1682
L5-120%	6441704	--
L6-150%75	8052131	

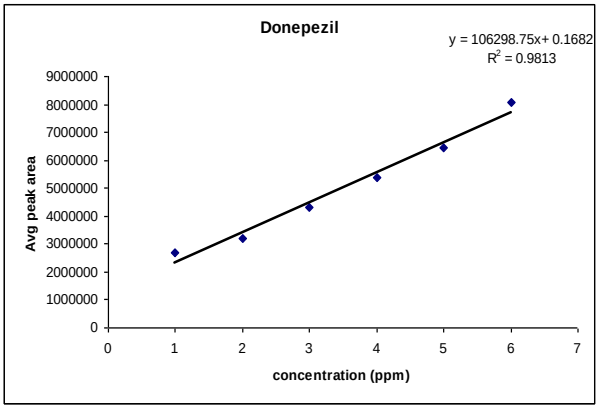

Fig 2: Linearity curve of Donepezil

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by using standard deviation of response and slope of the calibration curve. The LOD and LOQ of the proposed method was found to be 0.105 and 0.352 µg/ml respectively.

Assay Results:

The developed method has applied successfully for the analysis of Donepezil HCl in tablet dosage form. No further separation of API is required. Table 5 shows the results of % Assay.

%purity = At/As X Ds/Dt X Aw/ Label claim X p/100 X 100

Where,

At = Average area of sample
As = Average area of standard
Ds = Dilution factor of standard
Dt = Dilution factor of sample
Aw = Average weight of tablets taken for analysis in mg
P = Purity of working standard used

Table 3: Results of Assay

Tablet ID	Peak area	% Assay
1	1224800	99.6
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3	1216863	100.3
4	1221207	99.8
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6	1222697	99.2
Mean	1222697.33	99.26
SD	3750.47	0.86
%RSD	0.30	0.87

CONCLUSION:

Pharmaceutical analysis is the most important field, which includes usage of many sophisticated instruments, plays a key role factor for a dosage form to successfully come in to the market. In conclusion, the results indicating that the proposed method is precise, accurate, linear and robust for the determination of Donepezil HCl in bulk and its tablets. This method has the ability to separate the API from all the excipients with good resolution. It comply the method validation in line with ICH Guidelines. So it can be transferred from research environment to Quality Control environment to carry out the routine quality control testing.

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