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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DONEPEZIL HYDROCHLORIDE TABLETS BY RP-HPLC

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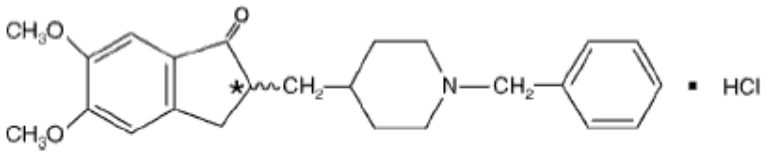
ABSTRACT

A simple, rapid and precise RP-HPLC method has been developed for the determination of donepezil hydrochloride in bulk and tablets. Separation was achieved with a keystone phenyl RP 250 × 4.6 mm i.d., 5 µM particle size analytical column using mixture of methanol, 0.02M phosphate Buffer (pH 7.5 ± 0.1) and triethylamine in the ratio 60: 40: 0.5 v/v as the mobile phase by Reverse phase isocratic mode. The instrumental settings are at a flow rate of 1mL/min, column temperature at 40°C and UV detection at 268 nm. The retention time was found to be 7.05 min. The correlation coefficient, RSD and tailing factor were found to be 0.9970, 0.002275 and 1.11 respectively. The percentage contents found by the proposed method were 100.14, 100.1 and 100.32 mg in three different brands. The proposed method can be used for the quantification of donepezil hydrochloride over linearity in the range of 50-150 µg/ml and the mean percentage recovery was found to be 100.37%. The intra day and inter day

precision were found 0.2034% and 0.2062 % respectively. This method can be used for clinical routine-monitoring/ *in vitro* metabolism studies and drug–drug interaction studies.

Keywords: Donepezil hydrochloride, HPLC, Isocratic, Validation

INTRODUCTION



2, 3-dihydro-5, 6-dimethoxy-2-[[1-(phenyl methyl)-4-piperidinyl] methyl]-1H-inden-1-one hydrochloride

USP-Donepezil hydrochloride RS (142057-77-0) is a cholinesterase inhibitor used to treat Alzheimer's disease where neurons are damaged in part of the brain that is involved in memory, learning, language and reasoning which may result from the deficiency of neurotransmitter, acetyl chorine. It is a white crystalline powder, freely soluble in methanol, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. It is a reversible inhibitor of the enzyme acetylcholinesterase known chemically as (±)-2, 3-dihydro-5, 6-dimethoxy-2-[[1-(phenyl methyl)-4-piperidinyl] methyl]-1*H*-inden-1-one hydrochloride. 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. This drug has been developed under USP's Pending Standards Guideline, and is not a USP–NF monograph[1].

It is not yet official in any pharmacopoeia. The literature survey revealed that a number of methods being reported for the estimation of donepezil hydrochloride in biological samples. There was no reported method for the estimation of the above drug in tablets by Reverse Phase HPLC method but several authors reported HPLC methods in biological samples[2-8]. We herein report a simple and reliable RP-HPLC method for the estimation of donepezil hydrochloride in bulk and pharmaceutical dosage forms.

MATERIAL AND METHODS

Reagents and chemicals

Pure standard of donepezil hydrochloride (99.91%) was obtained as a gift sample from ATOZ laboratories private limited, Chennai along with certificate of analysis (COA). Tablets of aricep 5 mg, dopezil 5 mg and aricep 10 mg were purchased from Apollo Pharmacy, Chennai and HPLC grade methanol, potassium dihydrogen phosphate, phosphoric acid, triethylamine and ethanol were purchased from Merck Company Mumbai. Hitachi, Merck HPLC instrument with a keystone phenyl RP 25 cm x 4.6 mm, 5µ analytical column was employed. Shimadzu precision analytical balance (0.001g sensitivity; model BL-220H) was employed. All the glasswares employed were cleaned using hot water, acetic anhydride and acetone and dried in hot air oven at 110° C. The working temperature was maintained between 18-21° C. Physical properties, TLC, melting point and IR studies confirmed the chemical structure and purity of the substance.

HPLC apparatus and chromatographic conditions

System configuration

System 1

Shimadzu Japan, detector: UV-11B VP, serial number: C_{18} 98411853 YU, pump: LC-10 AT VP, serial number: C_{18} 9841913556 N.

System 2

Hitachi, detector: UV-HR, serial number: TL_{20} 1567000915 YP, pump: LC-20 AT YP, serial number: L_{20} 19137208946 LP.

Separation achieved with a keystone phenyl RP 25 cm x 4.6 mm, 5 µ analytical column using methanol: 0.02 M Phosphate buffer: triethylamine in the ratio of 60: 40: 0.5 (pH 7.5 ± 0.1). The instrumental settings are at the flow rate of 1 mL/min, column temperature at 40° C and UV detection at 268 nm. The membrane (0.45µ) filtered and degassed (sonicated) sample (20µl) was injected in to the column. It was quantified by comparing the areas of both

standard and sample. Both sample and standard solutions (20 µl) were injected. The content in the sample was calculated from the mean area counts of three replicates for sample and standard preparations from the chromatograms. System suitability tests were carried out on five replicate injections of the standard solution containing the drug. Various parameters such as theoretical plates per meter, tailing factor, linearity, precision and recovery studies were carried out as per ICH guidelines[9].

Preparation of standard solution

Donepezil HCL (100µg/ml) was prepared by dissolving 50 mg of drug in little methanol and made up to 50 ml using methanol. Then 10 ml of above solution was used and made up to 100 ml using methanol.

Preparation of mobile phase

Methanol: 0.02M Phosphate buffer: triethylamine in the ratio of 60: 40: 0.5, pH 7.5 ± 0.1 Mixture was prepared and filtered through the membrane (0.45µ) and degassed before use.

Preparation of tablet sample

Film coat of ten tablets were removed with the help of a filter paper moistened with ethanol, weighed and crushed to obtain a fine powder. The powder equivalent to 5 mg of donepezil HCL was weighed and transferred to a 50 ml volumetric flask, 10 ml of mobile phase was added and the contents were shaken thoroughly and made up to 50ml using mobile phase to get the concentration of 100µg/ml of donepezil HCL. The amount present in each tablet was calculated by comparing the area of standard donepezil HCL and the tablet sample.

RESULTS AND DISCUSSION

The amount of drug present in each tablet for three replicates of donepezil HCL for all the three brands used in this investigation is presented in table 1. The retention time was found to be 7.05 min. The correlation coefficient, RSD and tailing factor were found to be 0.9970, 0.002275 and 1.11 respectively. The correlation co-efficient was found to be 0.9970.

Table 1: Showing the quantitation of donepezil HCL tablets

Label claim Amount found (mg)	% purity	t_R (min.)	Theoretical plates	Tailing factor (N) (mg)	Mean ± SD
Aricep- 5	5.007 ± 0.02	100.14	7.06	1523	1.11
Aricep- 10	10.01 ± 0.02	100.10	7.05	1524	1.12
Dopezil-5	5.016 ± 0.01	100.32	7.05	1524	1.12

*Mean of 3 replicates; The results of linearity study for the concentration (50-150 (µg/ml) showing peak area is given in table 2 and figure 1.

Table 2: Peak areas of donepezil HCL showing linearity

Concentration (µg/ml)	Peak area
50	559952
75	839923
100	1119853
125	1399871
150	1679838

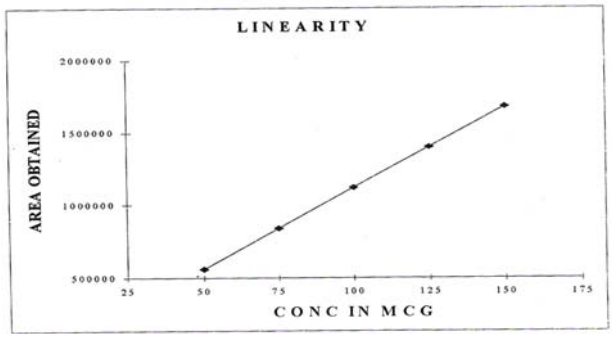


Fig. 1: Showing concentration and area linearity graph

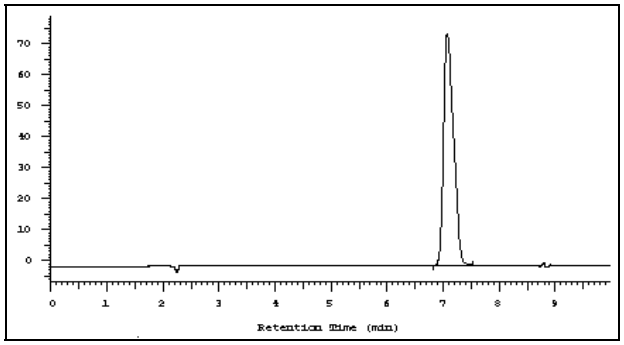


Fig. 2: Chromatogram showing the peak of standard drug

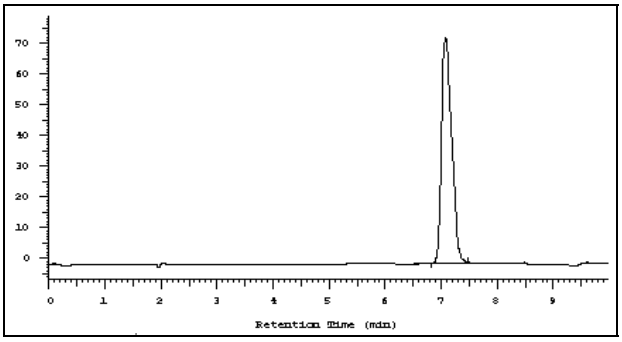


Fig. 3: Chromatogram showing the peak of aricep 5 mg tablets

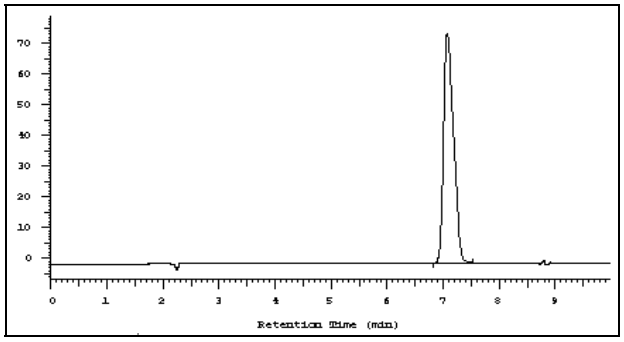


Fig.4: Chromatogram showing the peak of aricep 10 mg tablets

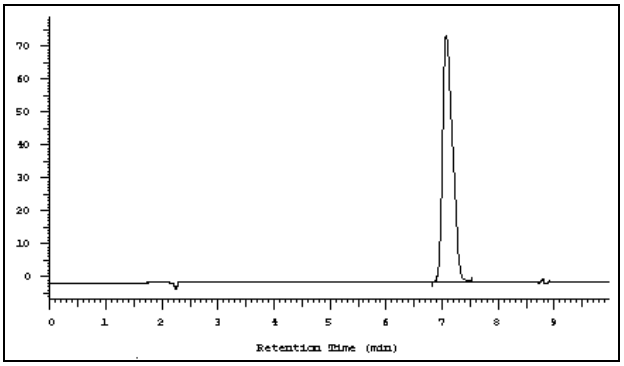


Fig. 5: Chromatogram showing the peak of dopezil 5 mg tablets

The HPLC chromatograms recorded for both standard and sample peaks are presented in figures 2-5.

Table 3: The % purity of donepezil HCL showing system and analyst parameters

Parameters	% purity Mean ± S.D.	% RSD
System –1	100.47 ± 0.01	0.011493
System –2	100.89 ± 0.02	0.02289
Analyst -1	100.15 ± 0.005	0.005765
Analyst –2	100.39 ± 0.01	0.01150

Recovery experiment was performed to study the accuracy, precision, and to check the interferences of excipients in tablets. Recovery of method was observed by the results from 3 placebo preparations accurately spiked with different concentrations (20, 40 and 50 µg/ml) of the active ingredient (donepezil HCL). The results are reported in table 4. The percentage recovery obtained was 100.53, 100.24 and 100.34 which indicated the good index of accuracy.

Table 4: Recovery results

Concentration (µg/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 deviation	--	0.1473
% RSD	--	0.1467%

Instrumental precision was established by repetitive injection of the same standard solution 10 times, followed by the averaging of the peak area and determination of the RSD (%) of all injections. The RSD (%) value obtained was below 1, which indicated the precision of the method. The Intra assay precision, mean % recovery and % RSD are 0.2034, 100.37 ± 0.147 and 0.146 respectively.

There was no interference of common excipients used in the tablet formulation. The percentage contents found by the proposed method were 100.14, 100.10 & 100.32 mg in aricep 5, aricep 10 and dopezil 5 mg tablets.

The elution of donepezil HCL was found to be extremely good at 268 nm at the flow rate of 1ml/min. It was also noted that the split peak was eliminated. The relationship between the standard amount taken and the area obtained for donepezil HCL is linear in the range (50-150 µg/ml) examined and all the points lie on a straight line. In recovery studies, it was observed that there was no significant difference between the label claim and actual amount and the method is shown to be accurate and selective. A relative standard deviation of less than 2% was obtained (0.1467%) which proved the accuracy of the method. The peak is reasonably symmetrical and high numbers of theoretical plates (1525) indicated the efficient performance of the column. These parameters represent specificity of the method. The method was statistically validated for linearity, accuracy, precision and selectivity following ICH recommendations. Due to its simplicity and accuracy, the method can be used for routine quality control analysis.

CONCLUSION

It can be concluded that the proposed HPLC method is accurate, precise, sensitive and reproducible for the analysis of donepezil hydrochloride in pharmaceutical dosage forms. The method was duly validated by the system suitability parameters and was found linear. This method can be used for clinical routine-monitoring/*in vitro* metabolism studies and drug-drug interaction studies.

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