NEW RP-HPLC METHOD FOR ESTIMATION OF DONEPEZIL HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple and sensitive HPLC method has been developed for the estimation of donepezil hydrochloride in pharmaceutical formulations. donepezil hydrochloride is a reversible inhibitor of acetyl cholinesterase, indicated for the treatment of mild to moderate dementia of the Alzheimer's type. The HPLC method was developed by using Phenomenex Luna C 18 column (250 mm length, 4.6 mm internal diameter and 5 µm particle size) and a 50 : 50 v/v mixture of acetonitrile and 0.025 M phosphate buffer (pH adjusted to 7.00 after addition of 5.0 mL of triethylamine) as a mobile phase. The analyte was monitored with UV detector at 228 nm. Typical retention time for donepezil hydrochloride was found to be 12.78 min. and the total run time for chromatography was maintained up to 18 min. The method was statistically validated for its linearity, accuracy and precision. Due to its simplicity and accuracy, the method can be used for routine quality control of donepezil hydrochloride in pharmaceutical formulations.

Key words : donepezil hydrochloride, C 18 column, HPLC method

INTRODUCTION

donepezil hydrochloride[1, 2], a piperidine derivative, is a reversible and specific inhibitor of acetylcholinesterase with actions similar to those of neostigmine. It is highly selective for the central nervous system and is used for the symptomatic treatment of mild to moderately severe dementia in Alzheimers disease. donepezil hydrochloride is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase. Chemically, donepezil hydrochloride is

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(±)-2, 3-dihydro-5, 6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl] methyl]-1H-inden-1-one hydrochloride. Literature survey revealed the availability of only a few analytical methods such as capillary electrophoresis[6,7], HPLC[8-12] LC-MS[13-15] and HPTLC[16] for estimation of donepezil hydrochloride in pharmaceutical formulations and in biological fluids. In the present investigation, a simple and accurate RP-HPLC method has been developed using RP- C 18 column and a mixture of acetonitrile and 0.025 M phosphate buffer (50 : 50 v/v) as a mobile phase.

EXPERIMENTAL

Material and methods

Instrumentation

Shimadzu HPLC (Class VP series) consisting of LC 10AT VP binary gradient pump, Phenomenex Luna C 18 column (250 mm length, 4.6 mm internal diameter and 5 μm particle size) and SPD 10A VP UV detector was used for chromatography. Systronics UV/VIS spectrophotometer 2201 was used for scanning the UV absorption spectrum. Elico LI 120 pH meter was used for adjusting the pH of the mobile phase. The data was acquired and analyzed with "Spincotech" software.

Chemicals used

donepezil hydrochloride was obtained as a gift sample from Sun Pharma India Limited. HPLC grade acetonitrile and triethyl amine were procured from Merck, Mumbai, India. Analytical reagent grade potassium dihydrogen orthophosphate and phosphoric acid were obtained from Qualigens, Mumbai, India. Water for HPLC was produced from Millipore apparatus.

Mobile phase preparation

A 25 millimolar phosphate buffer was prepared by dissolving 3.4 g of potassium dihydrogen orthophosphate in 1000 mL of water. To this, 5 mL of triethyl amine was added and pH was adjusted to 7.00 with orthophosphoric acid. Above prepared buffer and acetonitrile were mixed in the proportion of 50 : 50 v/v. The mobile phase so prepared was filtered through 0.22 μm nylon membrane filter and degassed by sonication.

Chromatographic conditions

The HPLC system consisting of Phenomenex Luna C 18 column (250 mm length, 4.6 mm internal diameter and 5 μm particle size) was stabilized with the mobile phase at a

flow rate of 1.0 mL/min. The test solutions were injected into the system by filling a 20 μL fixed volume loop manual injector. The chromatographic run time of 18 min. was maintained for the elution of the drug from the column. The eluates were monitored with UV detector at 228 nm.

Standard preparation

About 100 mg of pure donepezil hydrochloride was accurately weighed and dissolved in mobile phase in 100 mL volumetric flask to get 1 mg/ mL stock solution. A series of standard solutions in the concentration range of 2, 4, 6, 8, 10 and 12 μg/mL were prepared followed by a suitable dilution of stock solution with the mobile phase.

Sample preparation

About 20 tablets were taken and crushed to a fine powder. Tablet powder equivalent to 100 mg of donepezil hydrochloride was taken and transferred to 100 mL volumetric flask. About 70 mL of the mobile phase was added it and it was sonicated for 30 min. to extract the drug. The above sample mixture was filtered through Whatmann filter paper No. 1 into another 100 mL volumetric flask and diluted up to the mark with the mobile phase. It is further diluted to contain about 10 μg/mL of donepezil hydrochloride. Similarly blank was prepared by using the tablet excipients.

Procedure for estimation of the drug

Each standard solution of 2, 4, 6, 8, 10 and 12 μg/mL was injected thrice into the system followed by one blank injection and a calibration curve was constructed by plotting concentration of donepezil hydrochloride on X-axis and corresponding mean peak area on Y-axis. The sample prepared above was injected twice into the system and average peak area from chromatograms was determined. The concentration of the drug was computed from the calibration curve.

RESULTS AND DISCUSSION

After several systematic trials, the mobile phase consisting of mixture of 0.025M phosphate buffer (pH adjusted to 7.00 after addition of 5 mL of triethylamine) and acetonitrile in proportions of 50 : 50 v/v was fixed as a mobile phase.

The donepezil hydrochloride UV absorption spectrum was recorded in mobile phase. It shows four absorption bands at 204, 228, 267 and 312 nm. At 228 nm it is having maximum UV absorption and hence, this wavelength was chosen for detection of analyte
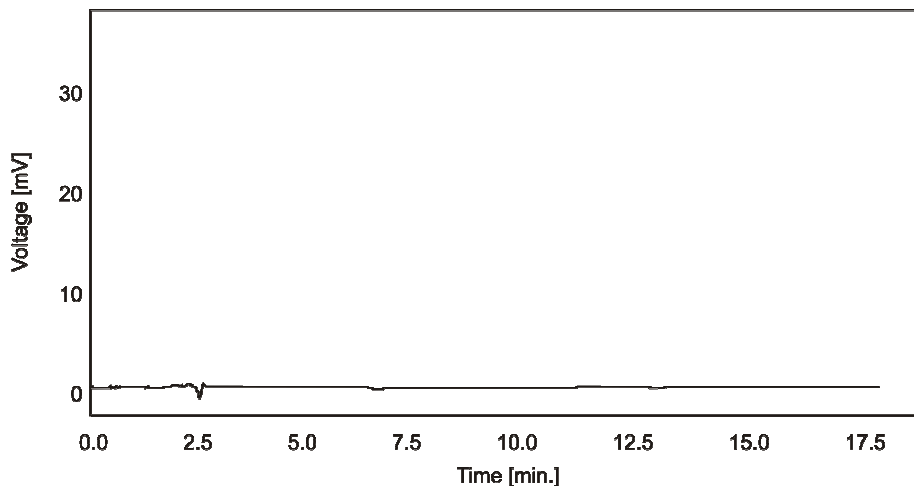
with UV detector.
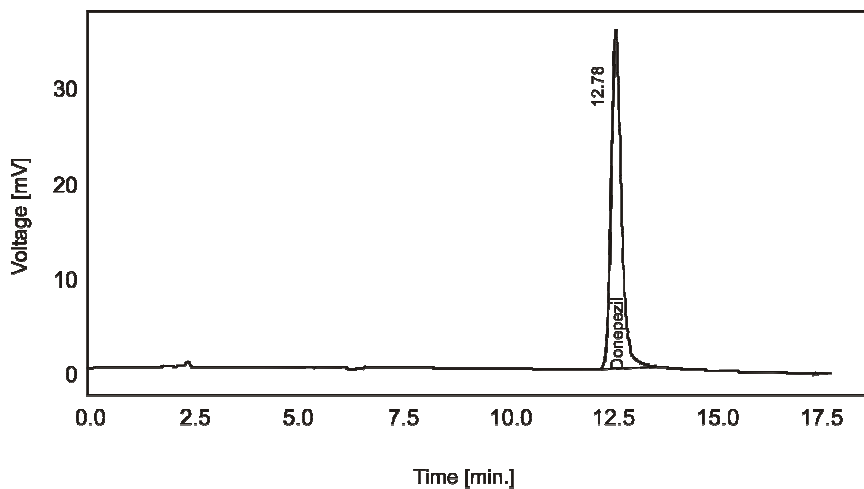

Fig. 1.Blank chromatogram

Fig. no. 2 : Typical test chromatogram

The blank chromatogram and sample chromatogram are given in Fig. 1 and 2, respectively. The drug elutes at a typical retention time of 12.78 min. No components were detected at the retention time corresponding to donepezil hydrochloride that indicates no interference of excipients from the tablet formulation. The method is having good linear response with correlation coefficient of 0.9997. The linearity graph is presented in

Fig. 3. The limit of detection and the limit of quantification of the method were found to be 0.1993 µg/mL and 0.6644 µg/mL, respectively.
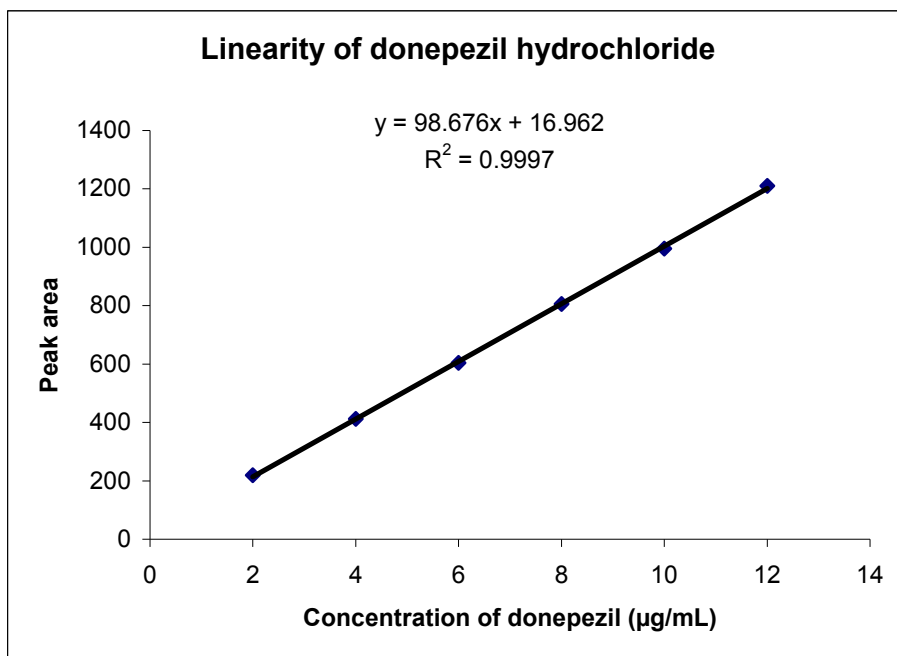

Fig. 3: Linearity graph of donepezil hydrochloride

Intra-day and inter-day precision was performed with 8 µg/mL and 10 µg/mL test solutions to study the day-to-day variations that may affect the chromatographic performance. The result was interpreted in terms of % RSD values. Low % RSD values were obtained indicating that the method is having good precision. The   intra-day and inter-day precision data are presented in Table 1.

Table 1. Precision of the method

donepezil HCl concentration (µg/mL)	Concentration of donepezil hydrochloride (µg/mL) found			
	Intra-day		Inter-day	
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
8	7.99	1.12	8.00	1.17
10	10.05	1.25	10.03	1.23

Table 2. Analysis of donepezil hydrochloride in pharmaceutical formulations

Formul-ation	Labeled amount (mg/ tablet)	Mean amount found (mg)*		Mean % recovery found	
		Proposed method (n=6)	Reference method[10] (n=6)	Proposed method	Reference method
Tablet 1	5	5.07 ± 0.08 t =1.33 F =3.28	5.06 ± 0.06 t = 0.987 F = 3.11	100.04 ± 0.20	100.02 ± 0.12
Tablet 2	10	10.11 ± 0.12 t = 1.11 F = 2.42	10.08 ± 0.11 t = 2.01 F = 2.59	99.99 ± 0.32	98.99 ± 0.21
Tablet 3	5	5.06 ± 0.09 t = 0.87 F = 2.32	5.05 ± 0.08 t = 1.21 F = 2.74	100.05 ± 0.16	99.95 ± 0.34
Tablet 4	10	10.05 ± 0.16 t = 1.93 F = 3.85	10.03 ± 0.15 t = 0.89 F = 3.27	99.99 ± 0.11	100.03 ± 0.28

* The "t" and "F" values refer to the comparison of test HPLC method with the reference HPLC method. Theoretical values of "t" and "F" at 95% level are 2.57 and 5.05, respectively.

The accuracy of the method was determined by adding known quantities of the drug to the previously analyzed formulations and reanalyzed by the proposed method. The accuracy of the method was supported by high recovery values obtained from the developed method. The % recovery results of the method are given in Table 2.

The developed method has been applied to the analysis of some commercially available tablet dosage forms. The results obtained from the proposed method and reference method[10] had been compared statistically. The results are in good agreement with each other as indicated by "t-test" and "F-test" results. The analysis results are incorporated in Table 2.

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