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Liquid Chromatographic Method for the estimation of Donepezil Hydrochloride in a Pharmaceutical Formulation

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Abstract : A stability indicating HPLC method for the estimation of donepezil hydrochloride in tablets was developed and validated. Donepezil hydrochloride is a reversible inhibitor of acetylcholinesterase, indicated for the treatment of mild to moderate dementia of the Alzheimer's type. The HPLC method was performed with a reversed phase C_{18} column (250 mm X 4.6 mm id, 5µm particle size), detection at 230 nm and a mixture of methanol, water and ortho phosphoric acid for pH adjustment at 4 (60:40) as mobile phase. Typical retention time for donepezil was 4.23 min. Forced degradation studies were carried out. The drug was found to be stable to the dry heat, photo-degradation, oxidation, basic, and acidic condition attempted which indicate drug is highly stable. Quantification was achieved with ultraviolet detection at 230 nm over the concentration range 2 – 60µg/ml with range of recovery 99.14 – 100.84 % for donepezil by the RP-HPLC 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.
Key Words: Validation; RP-HPLC; Donepezil hydrochloride.

Introduction

Donepezil (DONE), (6)-2-[(1-benzylpipereidin-4-yl)-methyl]-5,6-dimethoxy-indan-1-one monohydrochloride (Fig. 1), is a centrally and selectively acting acetylcholinesterase inhibitor and it exerts its therapeutic effect by increasing acetylcholine concentrations and enhancing cholinergic function. It is indicated for the treatment of mild to moderate dementia of the Alzheimer's type [1]. It has been reported that donepezil is effective in the treatment of cognitive impairment and memory loss in patients with Al- zheimer disease, and is well tolerated when 5 mg daily of the drug is prescribe [2].

All analytical methods found in literature are used to determine DONE in human plasma[3], [6]. Most of the methods cited in literature for DONE determinations involve HPLC[7], [10] and cyclic voltammetry using a glassy carbon electrode as the working electrode[11].

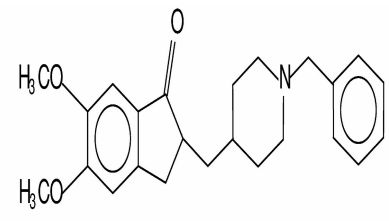

Fig. 1 Structure of Donepezil hydrochloride

On the other side, the stereoselective determination of DONE has been performed using HPLC[12] and capillary electrophoresis [13], [14]. The proposed method describes the development and validation of a stability-indicating method for the assay of DONE in tablets by HPLC and the procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

Experimental

Apparatus

A Series 200 HPLC system (PerkinElmer, Shelton, CT) equipped with a Series 200 diode array detector, Series 200 quaternary gradient pump, Series 200 column oven, manual injector rheodyne valve) with 20 µL fixed loop, Turbochrom navigator software (Version 6.1.1.0.0:K20), and Hypersil C18 column (150mm× 4.6mm id, 5µm particle size) was used.

Reagents and Materials

Analytically pure powder donepezil hydrochloride was procured as gratissamples from torrent pharmaceuticals Limited, Gujarat, India. HPLC grade water, methanol and orthophosphoric acid was purchased from E. Merck (Mumbai, India). Tablets containing donepezil HCL (ALZIL 5mg) of brand Intas Pharmaceuticals Ltd., Ahmedabad (Gujarat, India) were purchased from the local market.

Chromatographic Conditions

The Hypersil C_{18} column was used at ambient temperature. The mobile phase consisted of methanol; water (pH 4 adjusted with ortho-phosphoric acid) (60:40, v/v) and the flow rate was maintained at 1 ml/min. The mobile phase was passed through nylon 0.45µm– 47mm membrane filter and degassed before use. The elution was monitored with UV detector at 230 nm, and the injection volume was 20µL.

HPLC method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

Preparation of DONE Standard Stock Solutions (100µg/ml)

Accurately weighed 25mg of DONE transferred to a 25ml volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard solution of 1000µg/ml. This solution (1ml) was further diluted to 10 ml with mobile phase to obtain a working standard stock solution of 100µg/ml for the RP- HPLC method.

Preparation of Sample Solutions

Twenty tablets were weighed and finely powdered. A mass equivalent to 5mg of DONE was weighed and transferred in a 100ml volumetric flask, mixed with methanol (60ml), and sonicated for 20min. The solution was filtered through Whatman filter paper No. 41, and the residue was washed thoroughly with methanol. The filtrate and washings were combined in a 100ml volumetric flask and diluted to the mark with methanol. An aliquot of this solution 0.4ml was further diluted to 10ml with methanol to obtain a solution containing 20µg/ml of DONE and subjected to RP-HPLC analysis.

Method Validation

Linearity and range

Calibration curves were constructed by plotting peak areas versus concentrations of DONE, and the regression equations were calculated. The calibration curves were plotted over the concentration range 2–60µg/ml. Accurately measured standard working solutions of DONE (0.2, 0.5, 1.0, 2.0, 4.0, and 6.0ml) were transferred to a series of 10ml volumetric flasks and diluted to the mark with mobile phase. Aliquots (20µl) of each solution were injected under the operating chromatographic conditions described above.

Accuracy (recovery)

The accuracy of the method was determined by calculating recoveries of DONE by the standard addition method. Known amounts of standard solutions of DONE (50, 100, and 150%) were added to pre quantified sample solutions of tablets. The amounts of DONE were determined by applying these values to the regression equation of the calibration curve.

Method precision (repeatability)

The precision of the instruments was checked by repeatedly injecting (n = 6) solutions of DONE (20µg/ml) for the RP-HPLC method.

Intermediate precision

Precision was evaluated in terms of intraday and interday precision. The intraday precision was

investigated using different concentrations of standard solutions and sample solutions. The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses three times on the same day and on three different days over a period of 1 week for different concentrations of DONE standard and sample, respectively. The results were reported in terms of % RSD.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the effect on resolution was recorded. There was no detrimental effect on the method performance as shown. Low value of relative standard deviation was indicating that the method was robust.

LOD and LOQ

The LOD was determined by the analysis of samples with known concentrations of analyte and by establishing through visual evaluation the minimum level at which the analyte could be reliably detected. The LOQ was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be quantified with acceptable accuracy and precision.

Specificity

To assess the method specificity, synthetic formulation was prepared using excipients like starch, microcrystalline cellulose and talc. The synthetic formulation was analyzed for the interference of the excipients added.

Specificity was also assessed by performing forced degradation study. DONE is practically insoluble in water and is very soluble in methanol; therefore, methanol was used as the solvent in all studies. All solutions prepared for use in forced degradation studies were prepared to yield a starting DONE concentration of 10µg/ml.

(a) Oxidation: Solutions of DONE (10µg/ml) for oxidation studies were prepared using 3% H_2O_2 and 6% H_2O_2 in methanol in four separate volumetric flask, and the resultant solutions of two flask were stand for 24 hr and the other two volumetric flask reflux for 1 hr to facilitate oxidation of the DONE.

(b) Acid degradation: Solutions of DONE (10µg/ml) for acid degradation studies were prepared using 0.1M HCl, 1M HCl and 2M HCl in methanol in six different volumetric flask and the resultant solutions of three volumetric flask were stand for 24 hr and the other three volumetric flask reflux for 1 hr.

(**c**) Alkali degradation: Solutions of DONE (10µg/ml) for alkali degradation studies were prepared using 0.1M NaOH, 1M NaOH, 2M NaOH in methanol in six different volumetric flask and the resultant solutions of three volumetric flask were stand for 24 hr and the other three volumetric flask reflux for 1 hr.

(d) Dry heat: Solutions for dry heat studies were prepared by exposing powder to dry heat (80^{o}C) in an oven for 2 days. The powder was removed from the oven, and DONE was accurately weighed and transferred to a volumetric flask to give a final DONE concentration of 10µg/ml.

(**e**) Sunlight radiation.: Solutions of DONE (10µg/ml) in methanol for photolytic study were prepared by exposing powder to sunlight (35^{o}C) to determine the effects of light irradiation on the stability of DONE. Samples were placed in direct sunlight for 24 hr. The sample was removed from the sunlight, and solutions were prepared for analysis as previously described.

System suitability

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of DONE to be performed. The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values with the acceptance criteria of the U.S. Food and Drug Administration, Center for Drug Evaluation and Research guidance document (U.S. Food and Drug Administration, 1994). A system suitability test of the chromatography system was performed before each validation run. Six replicate injections of a system suitability/calibration standard and one injection of a check standard were made. Area, retention time (RT), tailing factor, asymmetry factor, and theoretical plates for the six suitability injections were determined.

Stability of standard and sample solutions

Stability of standard and sample solution of DONE was evaluated at room temperature for 48 hr. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were stable up to 48 hr at room temperature.

Determination of DONE in Tablets

Tablets of DONE were purchased from a local market. The responses of tablet solutions measured with the UV detector showed a wavelength maximum at 230 nm for the RP-HPLC method. The amounts of DONE present in sample solution were determined by fitting the responses into the regression equation for DONE.

TABLE 1 :SYSTEM SUITABILITY TEST PARAMETERS FOR DONEPEZIL HYDROCHLORIDE THE PROPOSED HPLC METHOD

Parameter	Donepezil HCl
Retention times (R_T)	4.23 Min
HPLC Plate Count	6394
Tailing factor	1.67
Base width (sec)	15.98

TABLE 2: REGRESSION ANALYSIS OF CALIBRATION GRAPHS FOR DONEPEZIL HYDROCHLORIDE BY PROPOSED HPLC METHOD

Parameter	Donepezil HCl
Linearity (µg/ml)	2 – 60
Correlation co –efficient (r)	0.998
Slope of Regression(S)	61728
Intercept of Regression	32196
Standard deviation of slope	406.92
Standard deviation of intercept	4313.55

TABLE 3 :DATA DERIVED FROM ACCURACY OF DONEPEZIL HYDROCHLORIDE THE PROPOSED HPLC METHOD

Amount of Sample (µg/ml)	Sets	Amount drug of spiked (µg/ml)	Area(n= 3)	Average amount recovered (µg/ml)	% Recovery	Mean % Recovery	% RSD
20	1	0	1214134	20.17	100.95	100.84	0.51
	2	0	1218266		101.25		
	3	0	1206195		100.31		
20	1	10	1809446	29.96	99.17	99.78	0.58
	2	10	1812322		99.41		
	3	10	1828934		100.75		
20	1	20	2427863	39.83	99.27	99.14	0.31
	2	20	2432835		99.67		
	3	20	2418176		98.48		
20	1	30	3064637	49.99	100.85	99.99	0.35
	2	30	3043405		99.13		
	3	30	3053972		99.98		

TABLE 4: SUMMARY OF VALIDATION PARAMETERS FOR DONEPEZIL HYDROCHLORIDE THE PROPOSED HPLC METHOD

Parameters	Donepezil HCl
LOD (µg/ml)[a]	0.5
LOQ (µg/ml)[b] n=5	2.0
Accuracy, %	99.14 – 100.84
Repeatability, (% RSD, *n* = 6)	0.14 – 0.80
Precision (% RSD)	
Inter day (n = 3)	0.19 – 0.89
Intra day (n = 3)	0.23 – 0.50

[a]LOD = Limit of detection.,[b]LOQ = Limit of quantitation.

TABLE 5: DATA DERIVED FROM ROBUSTNESS OF DONEPEZIL HYDROCHLORIDE THE PROPOSED HPLC METHOD

Parameters	Normal condition	Change in condition	Change in % RSD
Flow Rate	1.0 ml/min	0.9 ml/min	0.45
		1.1 ml/min	0.41
pH	4.0	3.5	0.22
		4.5	0.32
Mobile phase ratio	60:40	65:45	0.38
		55:45	0.31

Result and Discussion

Optimization of the chromatographic condition

Several mobile phases were tried to resolve DONE but the resolution was not satisfactory. So modification was made in the above mobile phase. Finally the system containing methanol: water (pH 4 adjusted with orthophosphoric acid) (60:40, v/v) as the mobile phase at a flow rate of 1.0ml/min was found to be satisfactory and gave well resolved peak for DONE. The retention time for DONE was 4.23 min. For the selection of detection wavelength, the spectrum of 10 ppm DONE revealed that, at 230 nm the drug possesses significant absorbance. So considering above fact, 230 nm was selected as a detection wavelength for estimation of DONE using HPLC. Complete resolution of the peaks with clear baseline separation was obtained (Figure 2). The system suitability test parameters are shown in Table 1.

Validation of the Proposed Method

The developed method was validated, as described below, for various parameters like linearity and range, accuracy, precision, ruggedness, system suitability, specificity, LOQ and LOD.

Linearity of the method was evaluated at six concentration levels by diluting the standard stock solution to give solutions in the range of 2.0– 60µg/ml. The calibration curve for DONE was prepared by plotting area v/s concentration. Calibration data for DONE was shown in Table 2. The linearity plot of DONE was found to be linear with the linear equation y = 61728x – 32196 and correlation coefficient 0.997 for DONE. Linearity was observed in the expected concentration range, demonstrating suitability of the method for analysis. This indicates that the method is linear in the specified range for the analysis of DONE in dosage form. The recovery experiments were carried out by the standard addition method. The method was found to be accurate with % recovery 99.15% – 100.85% and has found with acceptable %RSD of not more than 2% at each level. The recoveries obtained by the RP-HPLC method for DONE are shown in Table 3. Precision was calculated as repeatability and intraday and interday variation for DONE. The method was found to be precise with CV=0.38 for intraday (n=3) and CV =0.43 for interday (n=3) for DONE. The low value of CV (i.e. NMT 2%) has observed for the three results shown in Table 4 hence it concluded that the method is precise for the analysis of DONE in their dosage form. There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of DONE in their dosage form. The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in Table 5. The low value of relative standard deviation was indicating that the method was robust. Standard and sample solution stability was evaluated at room temperature for 24 hr. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were stable up to 24 hrs at room temperature. These data shows, the method was found to be sensitive for the determination of DONE. The LOD and LOQ were measured by a visual method and were found to be 0.5 and 2.0µg/ml, respectively.

Degradation Behavior of DONE

Forced degradation study was carried out by subjecting the drug to acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photolytic (sun light) conditions. The DONE was found to be stable to oxidative stress degradation, dry heat degradation, photolytic condition, acid and alkali hydrolysis.

The degradation study was carried out for oxidative stress degradation using 3% and 6% H_2O_2, 2M HCl for acid hydrolysis and 2M NaOH for alkali hydrolysis. Dry heat degradation was performed at 80^0C for 2 hr. The study indicated that DONE was highly stable to

chemical oxidation study, dry heat, photolytic condition, acid, and alkali hydrolysis.

Analysis of marketed formulation

The proposed method was applied for the determination of DONE in tablets of donepezil HCl. The results of these assays was 99.84% (n=3) (RSD = 0.21%) of the label claim for the formulation. The results of the assay indicated that the method is selective for the assay of DONE without interference from excipients used in the tablets.

Conclusions

A validated stability-indicating HPLC analytical method has been developed for the determination of DONE in bulk and in tablet dosage form. The results of stress testing undertaken according to the ICH guidelines revealed that the method is selective and stability-indicating. The proposed method is simple, accurate, precise, and specific, and it has the ability to separate the drug from degradation products and excipients found in the dosage form but from all stability conditions the DONE was found to be highly stable molecule. The method is suitable for the routine analysis of DONE in tablets. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

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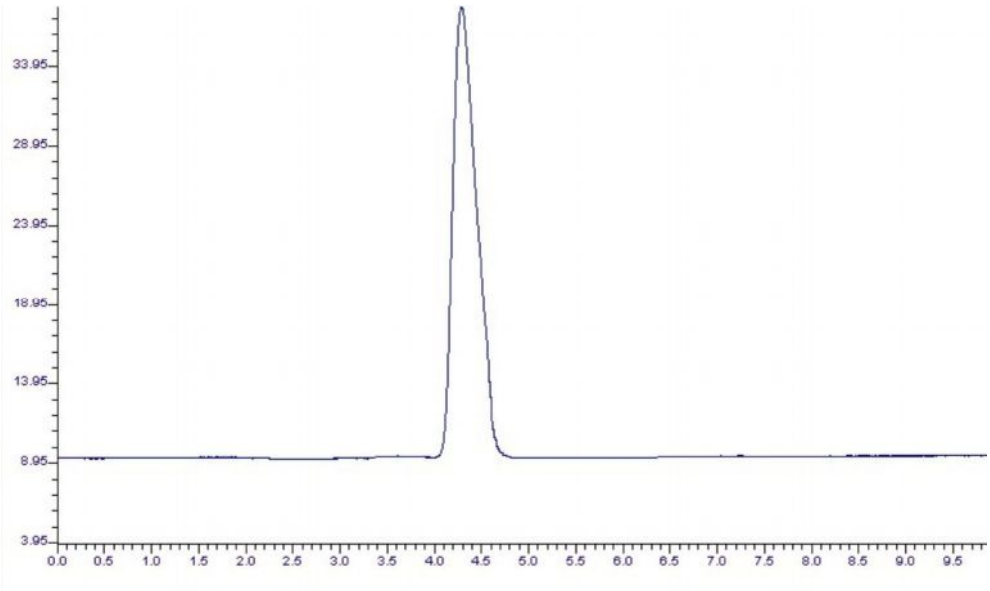

Fig. 2 Chromatogram showing peak of Donepezil hydrochloride

HPLC chromatogram of Donepezil hydrochloride (R_T 4.23 min) on C_{18} hypersil column using methanol: water (pH 4 adjusted with orthophosphoric acid) (60:40, v/v) as the mobile phase

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