RP-HPLC Method for the Estimation of Donepezil Hydrochloride Dosage Form

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Abstract: A simple and accurate methods to determine donepezil, in tablet dosage form, were developed and validated using liquid chromatography (LC). The LC separation was achieved on a Inertsil C8-3, 25 cm x 4.6-mm, 5 μ in the isocratic mode using buffer : methanol : triethylamine (550:450:5)v/v, adjusted to pH 2.50 $\pm$ 0.05 with orthophosphoric acid, as the mobile phase at a flow rate of 1.0 mL/min. The methods were performed at 271 nm. In LC method, quantification was achieved with PDA detection over the concentration range of 20-60 μ g/mL with mean recovery of 100.18 $\pm$ 0.75%. The method was validated by determining its sensitivity, accuracy and precision. The proposed method is simple, fast, accurate and precise and hence can be applied for routine quality control of donepezil HCl in bulk and tablet dosage form.

Keywords: Donepezil HCl, RP-HPLC, Estimation and Tablets.

Introduction

Donepezil HCl ARICEPT® (donepezil hydrochloride) is a known chemically as ($\pm$)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one hydrochloride (also known as E2020 or Aricept®). It has an empirical formula of C₂₄H₂₉NO₃HCl and molecular weight of 415.96. The registered trademark of Eisai Co. Ltd, Tokyo, Japan) is a piperidine based inhibitor of the enzyme acetylcholinesterase (AChE). It has recently been approved for marketing in the USA, Canada and several EU member states, including the UK, for the symptomatic treatment of mild to moderate Alzheimer's disease. *In vitro* studies have demonstrated that donepezil has a significantly greater degree of selectivity for AChE in the central nervous system (CNS) than for butyrylcholinesterase (BuChE) in the periphery. Clinical trials undertaken in the USA and Europe have demonstrated that donepezil

(5 mg or 10 mg, once daily) significantly improves cognitive and global function in patients with Alzheimer's disease. Furthermore, these studies have shown that donepezil is well tolerated and is not associated with the hepatotoxicity that is commonly seen with acridine-based cholinesterase inhibitors, such as tacrine. Phase I studies conducted in the USA have demonstrated that donepezil pharmacokinetics are linear and dose proportional and are characterized by slow plasma clearance and a long half-life (70–80 h). Although donepezil is metabolized primarily by the P-450 isoenzyme CYP-3A4 and to a lesser extent by CYP-2D6, compromised hepatic function does not significantly affect its pharmacokinetic profile. Donepezil hydrochloride is a white crystalline powder and is freely soluble in chloroform, 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.

Since its recognition as a broncodilator almost 50 years ago, theophylline, a methylxanthine alkaloid, has played an important role in the management of both acute and chronic reversible airway obstruction, in particular asthma and chronic bronchitis. However, it has a narrow safety window, which, along with its variability in disposition, makes dosing difficult to predict and toxicity difficult to prevent. Symptoms of toxicity include tachycardia, severe restlessness, agitation, nausea and vomiting, particularly at high blood concentrations.

The physical status of a patient can greatly alter theophylline metabolism and/or elimination, resulting in toxic plasma levels. Several disease states, as well as advanced age, smoking and obesity, have been shown to influence the pharmacokinetics of theophylline. Previous reports have also demonstrated that the half-life of theophylline is prolonged, and plasma clearance decreased, in patients with liver disease, acute pulmonary oedema and congestive heart failure.

Theophylline is prone to clinically significant interactions with other drugs. For instance, the clearance of theophylline is increased substantially during the concomitant administration of phenytoin. Rifampin and oral contraceptives produce smaller but appreciable increases in theophylline clearance, whereas cimetidine and erythromycin have been reported to reduce the clearance of theophylline.

Experimental

Donepezil Tablets (ARICEPT 1; 5 mg/tablet, ARICEPT 2; 5 mg/tablet) of Zydus Cadila Ltd., (Ahmedabad, India) were purchased from local market. HPLC grade methanol were purchased from SD fine chemical (Ahmedabad, India). The water for LC was prepared by triple glass distillation and filtered through nylon 0.45 μ m-47 mm membrane filter (Gelman laboratory, Mumbai, India). Potassium dihydrogen orthophosphate, orthophosphoric acid and triethylamine were procured from SD fine chemical (Ahmedabad, India) and were of analytical grade.

Instrument

High Performance Liquid Chromatograph, Waters Alliance HPLC 2996, instrument equipped with PDA detector 2996, Auto injector of 200 μ L loop was used with Empower software. A Sartorius CP224S (Gottingen, Germany) analytical balance, and an ultra sonic cleaner (Frontline FS 4, Mumbai, India) were also used.

Chromatographic conditions

Chromatographic separation were achieved using a Inertsil C8-3, 25-cm x 4.6-mm, 5 μ GL sciences Japan, in the isocratic mode using buffer: methanol: Triethylamine (550:450: 5)v/v, adjusted to pH 2.50 $\pm$ 0.05 with orthophosphoric acid, as the mobile phase at a flow rate of 1.0 mL/min. The mobile phase was filtered through nylon 0.45 μ m-47 mm membrane filters and was degassed before use. The elution was monitored at 271 nm and the injection volume was 20 μ L. and column oven temperature 50 $^{\circ}$ C.

Preparation of buffer

2.75 g of potassium dihydrogen orthophosphate was transferred into 1000 mL water. Sonicated to dissolve and then adjusted the pH to 2.50 $\pm$ 0.05 by adding orthophosphoric acid. Then filtered through nylon 0.45 μ m-47 mm membrane filter and degassed prior to use.

Preparation of donepezil standard solutions

Accurately weighed 50.0 mg of donepezil hydrochloride was transferred to a 250 mL volumetric flask, add about 150 mL of mobile phase and sonicated to dissolve, diluted up to the mark with mobile phase and mixed. Further 5 mL of the resulting solution was diluted to 25 mL with mobile phase.

Preparation of donepezil sample solutions

Accurately not less than 10 tablets were weighed and crushed to fine power. Powdered tablets were accurately weighed (20 mg of donepezil hydrochloride) and transferred into a 100 mL volumetric flask. About 60 mL of mobile phase was added and sonicated for 10 min with intermittent shaking. Further diluted up to the mark with mobile phase and mixed. The resulting solution was centrifuged at 3000 rpm for 15 min. Further 5 mL of the clear supernatant solution was diluted to 25 mL with mobile phase.

Method validation*Specificity**(a) No interference from excipients*

This was demonstrated by injecting blank, placebo, sample and standard prepared from the same. There is no peak at the retention time of donepezil. There by indicating that there was no interference form blank and excipients.

(b) No interference from degradation products.

This was demonstrated by carrying out forced degradation of sample blend with 5N HCl, 5N NaOH, 30% H₂O₂, heating in water bath at 80 $^{\circ}$ C and keeping under UV light at 254 nm for 24 h. Prepared samples as per test preparation was injected into HPLC with photodiode array detector. In each case, purity factor for donepezil peak was determined to examine interference from degradation products.

Acid degradation

Accurately weighed quantity of powdered tablets equivalent to 20 mg of donepezil hydrochloride was transferred to a 100 mL volumetric flask. 10 mL of mobile phase was added and sonicated for 10 min. 5 mL of 5N HCl was added and heated in water bath at 80 $^{\circ}$ C for 30 min. Cooled and neutralized with 5 mL of 5N NaOH, volume was made up with mobile phase. This solution was injected into the HPLC system. The purity angle was less than purity threshold for donepezil peak.

Alkali degradation

Accurately weighed quantity of powdered tablets equivalent to 20 mg of donepezil hydrochloride was transferred to a 100 mL volumetric flask. 10 mL of mobile phase was added and sonicated for 10 min. 5 mL of 5N NaOH was added and heated in water bath at 50 °C for 30 min. Cooled and neutralized with 5 mL of 5N HCl, volume was made up with mobile phase. The resulting solution was centrifuged at 3000 rpm for 15 min. 5 mL of the supernatant solution was diluted to 25 mL and volume was made with mobile phase. This solution was injected into the HPLC system. The purity angle was less than purity threshold for donepezil peak.

Peroxide degradation

Accurately weighed quantity of powdered tablets equivalent to 20 mg of donepezil hydrochloride was transferred to a 100 mL volumetric flask. 10 mL of mobile phase was added and sonicated for 10 min. 2 mL of 30% H₂O₂ was added and heated in water bath at 80 °C for 30 min. Cooled and neutralized with 5 mL of 5N HCl, volume was made up with mobile phase. The resulting solution was centrifuged at 3000 rpm for 15 min. 5 mL of the supernatant solution was diluted to 25 mL and volume was made with mobile phase. This solution was injected into the HPLC system. The purity angle was less than purity threshold for donepezil peak.

UV Degradation

Accurately weighed quantity of powdered tablets equivalent to 20 mg of donepezil hydrochloride (Previously kept in UV light for 24 h) was transferred to a 100 mL volumetric flask. 60 mL of mobile phase was added and sonicated for 10 min. The volume was made up with mobile phase. The resulting solution was centrifuged at 3000 rpm for 15 min. 5 mL of the supernatant solution was diluted to 25 mL and volume was made with mobile phase. This solution was injected into the HPLC system. The purity angle was less than purity threshold for donepezil peak.

Thermal degradation

Accurately weighed quantity of powdered tablets equivalent to 20 mg of donepezil hydrochloride was transferred to a 100 mL volumetric flask. 20 mL of mobile phase was added and sonicated for 10 min. Then heated in a water bath at 80 °C for 30 min. Cooled and volume was made with mobile phase. The resulting solution was centrifuged at 3000 rpm for 15 min. 5 mL of the supernatant solution was diluted to 25 mL and volume was made with mobile phase. This solution was injected into the HPLC system. The purity angle was less than purity threshold for donepezil peak.

Calibration curve (Linearity)

LC method

The Calibration curves were plotted over the concentration range of 20-60 µg/mL. Accurately measured working standard solutions of donepezil (2.5, 4.0, 5.0, 6.0 and 7.5 mL) were transferred in to a series of 25 mL volumetric flasks and diluted to the mark with diluent. 20 µL of each solution were injected under the operating chromatographic conditions as described above. Calibration curves were constructed by plotting peak areas vs. concentrations of donepezil, and the regression equations were calculated. Each reading was average of three determinations.

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of donepezil by the standard additions method. Known amount of standard solution of donepezil (50%, 100% and 150% of label claim) for LC method, were added to a pre-quantified sample solution of tablet dosage form. The amount of donepezil was estimated by applying these values to the regression equation of calibration curve.

Method precision (Repeatability)

The precision was checked by repeatedly injecting ($n = 6$) standard solution of TAD ($1.5 \mu\text{g/mL}$) for LC method.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines (41).

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where σ = the standard deviation of the response; S = the slope of the regression line

Analysis of donepezil tablet dosage form

Tablets containing donepezil were purchased from local market. The responses of the tablet dosage form were measured at 271 nm for quantification of donepezil by LC. The amount of donepezil present in sample solution was determined by fitting the responses into the regression equation of donepezil.

Robustness

Three sample solutions of same lot of donepezil shall be prepared as per method and analyzed using different chromatographic condition as below.

- (1) Change in flow ($\pm 0.2 \text{ mL/min}$)
- (2) Change in organic composition of mobile phase ($\pm 5.0\%$).
- (3) Change in pH of mobile phase by ± 0.2 unit.
- (4) Change in temp of column by ± 0.2 unit.

Stability in analytical solution

This was evaluated by injecting initially a freshly prepared donepezil standard solution and sample solution at different time intervals. The peak response data is given below (Table 1)

Table 1. Peak response data.

Time, h	Ratio of peak response		% Deviation From Mean Initial Area Counts (Limit: $\pm 3.0\%$)	
	Standard	Sample	Standard	Sample
Initial	1302095	1247814	0.0	0.0
12	1306036	1255382	0.3	0.6
24	1308838	1256448	0.5	0.7
36	1311178	1263092	0.7	1.2
48	1316012	1265641	1.1	1.4

Results and Discussion

LC method

To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for donepezil was obtained with mobile phase consisting of buffer: methanol: triethylamine (550: 450: 5) v/v, adjusted to $\text{pH } 2.50 \pm 0.05$ with orthophosphoric acid, as the mobile phase at a flow rate of 1.0 mL/min . The elution was monitored at 271 nm. Better resolution of the peaks with clear base line separation was found (Figure 1).

Validation of the proposed methods

Linearity

Linear correlation was obtained between peak area and concentration of donepezil in the range of 20-60 µg/mL for LC method. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression ($r = 0.9999$). The regression analysis of the calibration curves is shown in Table 2.

Figure 1. System precision chromatogram.

Table 2. Regression analysis of the calibration curves for donepezil hydrochloride by the proposed LC method.

Parameter	LC method
Linearity range, µg/mL	20.8-60.84
Correlation coefficient, r	0.9996

Accuracy (% Recovery)

The recovery study was carried out by the standard addition method. The percent mean recoveries obtained were $100.18 \pm 0.75\%$ (Table 3) which was satisfactory. Repeatability was evaluated in terms of percentage relative standard deviation (%RSD), which was found to be 1.72%. The method is also found to be robust as no change was found in results after changing the chromatographic conditions. The system suitability test parameters are listed in Table 4.

Table 3. Summary of validation parameters for donepezil hydrochloride by the proposed LC method.

Parameter	LC method
LOD ^a , µg/mL	0.04
LOQ ^b , µg/mL	0.1
Accuracy, %	100.18
RSD %	1.72

^a LOD = Limit of detection; ^b LOQ = Limit of quantification; ^c RSD = Relative standard deviation

Table 4. System suitability parameters for donepezil hydrochloride by the proposed LC method.

Parameter	donepezil $\pm$ % RSD ^a
Retention time, min	8.52 ± 0.01
USP Tailing factor	1.13 ± 0.02
USP Plate Count	7390.48

^a RSD = Relative Standard deviation.

Method Precision

The percentage relative standard deviation (% RSD) for donepezil was found to be 1.15 % by LC method (Table 3). The RSD values indicate the proposed methods are repeatable.

Limit of detection and limit of quantification

The LOD and LOQ of donepezil were found to be 10.000 and 0.0400 µg/mL, respectively by LC. The validation parameters are summarized in Table 3. The proposed method is found to be specific, as no interference of excipients was found in the estimation of donepezil in tablet dosage form.

Analysis of donepezil tablet dosage form

The proposed methods were successfully applied to determine donepezil in tablet dosage form. The results obtained for donepezil were comparable with the corresponding label claim. (Table 5).

Table 5. Analysis results for donepezil hydrochloride tablets by the proposed LC method.

Sample ID	Donepezil HCl ± SD ^a (n ^b =5),
	LC
S1 (ARICEPT 5 mg)	101.32
S2 (ARICEPT 5 mg)	101.44

^a SD = Standard deviation; ^b n = Number of determinations

Conclusion

The results of the analysis of tablet dosage forms by the proposed methods are reproducible, reliable and are in good agreement with the label claim of the drug. Method were validated and found to be simple, sensitive, specific, accurate and precise. Statistical comparison of the assay results for donepezil in tablet dosage forms by method indicated no significant difference. The excipients present in the tablet dosage form did not interfere with determination of donepezil. Hence, method can be used successfully for the routine analysis of donepezil in tablet dosage form of donepezil.

References

1. Iimura Y, Mishima M and Sugimoto H, *J Label Comp Radiopharm.*, 2006, **27**, 835–839.
2. Rogers S L, Yamanishi Y and Yamatsu K, E2020-The pharmacology of a piperidine cholinesterase inhibitor. In: Becker R, Giacobini E; Editors, *Cholinergic Basis for Alzheimer Therapy*. Boston: Birkhäuser, 1991, 314–320.
3. Sherman K, Pharmacodynamics of oral E2020 and tacrine in humans: Novel approaches. In: Becker R, Giacobini E; Editors, *Cholinergic Basis for Alzheimer Therapy*. Boston: Birkhäuser, 1991, 321–328.
4. Rogers S L and Friedhoff L T, *Dementia*. 1996, **7**, 293–303.
5. Rogers S L and Friedhoff L T, *Eur Neuropsychopharmacol.*, 1998, **8**, 67–75.
6. Rogers S L, Doody R S, Mohs R and Friedhoff L T, *Arch Intern Med.*, 1998, **158**, 1021–1031.
7. Rogers S L, Farlow M R, Doody R S, Mohs R and Friedhoff L T, *Neurology*, 1998, **50**, 136.
8. Watkins P B, Zimmerman H J and Knapp M J, *JAMA.*, 1994, **271**, 992–998.
9. Rogers S L, Cooper N M, Sukovaty R, Pederson J E, Lee J N and Friedhoff L T, *Br J Clin Pharmacol.*, 1998, **46**(Suppl. 1), 7–12.
10. Rogers S L, Walters E J and Friedhoff L T, *Neurobiol Aging.*, 1992, **13**(Suppl.1), 125–126.
11. Rogers S L and Friedhoff L T. *Clin Pharmacol Ther.* 1997, **61**, 181 (PII–63).
12. Tiseo P J, Vargas R, Perdomo C A and Friedhoff L T, *Br J Clin Pharmacol.*, 1998, **46**(Suppl. 1), 51–55.
13. Serafin, W E. Drugs used in the treatment of asthma. In: Gilman A G, Molinoff P B, Ruddon R W, Hardman J G and Limbird L E; Editors, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 9th Ed., New York: McGraw-Hill, 1995, 659–682.
14. Griffin J P, D'Arcy P F and Speirs C J., *Manual of Adverse Drug Interactions*. 4th Ed., London, John Wright, Theophylline and derivatives, 1988, 427–439.
15. Reynolds, J E F, Martindale, *The Extra Pharmacopoeia*, 31st Ed., London, The Pharmaceutical Press, 1996, 1657–1665.