



Quantitative determination of donepezil hydrochloride by a simple and accurate synchronous spectrofluorimetric method in human plasma

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ABSTRACT

Aim: The aim of the study was to develop and validate a new simple, accurate, sensitive and specific bioanalytical spectrofluorimetric method for quantitative determination of donepezil hydrochloride (DH) in human plasma. **Method:** Estimation of DH in human plasma was done by spiked human plasma studies. Foreestimation of DH in human plasma extraction was carried out using 5% TCA for protein precipitation followed by liquid-liquid extraction with 5% IPA in n-Hexane. Under spectrofluorimetric conditions delta value 65 applied in synchronous mode (medium sensitivity mode) and wavelength used in analysis was 389 nm. **Result:** Developed method found linear, in the concentration range of 50-105 ng/mL with correlation coefficient (R^2) 0.9965. Percentage recovery was found to be 75 %-85 % for DH. High recovery show that the method is free from the interference from plasma constituents. **Conclusion:** Proposed method can be used for estimation of DH in routine quality control laboratories for quantitative determination of DH in bulk as well as in human plasma. Further it can also be used to determine plasma DH concentration in drug monitoring or in pharmacokinetic investigations.

Key words: Bioanalytical method, donepezil hydrochloride, human plasma, synchronous spectrofluorimetry

INTRODUCTION

Donepezil is chemically (RS)-2-[(1-benzyl-4-piperidyl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one.^{1,2} Donepezil hydrochloride (DH) is a specific and reversible inhibitor of acetylcholinesterase, and used in the treatment of Alzheimer's disease³ (Figure 1).

Literature review of DH reveals that *ultraviolet* (UV)-visible spectrophotometry method in tablet dosage form,⁴ HPLC methods in tablets,^{5,6} HPLC methods in human plasma,⁷ HPLC method in human and rat plasma, blood and microdialysates,⁸ liquid chromatography-mass spectrometry (LC-MS)-MS method,⁹⁻¹¹ HPTLC method in tablet dosage form¹² and enantiomers separation by HPLC method and its application in pharmacokinetics¹³ methods were being reported for the estimation of DH in biological samples and in tablet dosage form.

However, there is no method reported till now for the estimation of DH in human plasma by spectrofluorimetric method. The aim of this work was to develop a validated,

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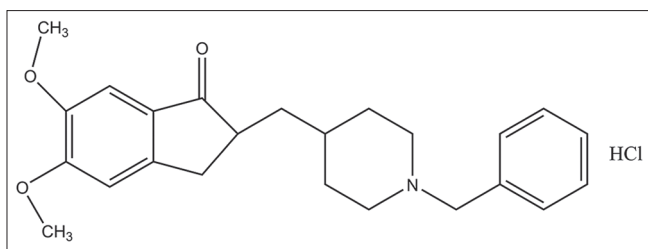


Figure 1: Structure of donepezil hydrochloride

sensitive, specific and simple, accurate, and time saving spectrofluorimetric method for the determination of DH in human plasma.

MATERIALS AND METHODS

Instrument

Spectrofluorimeter Model: FP-6500 PC series, Matched quartz cell (1 cm), Wavelength range: 220.00-750.00 nm, JASCO Japan.

Chemicals and reagents

API of DH was kindly gifted by INTAS Pharmaceuticals, Ahmedabad, Gujarat. Boric Acid, potassium chloride, sodium hydroxide, trichloroacetic acid (TCA), perchloric acid, N-Hexane, Iso-Propyl Alcohol, (AR Grade, S.D. Fine Chemicals Ltd., Mumbai, India) were used. Blank Human Plasma was kindly gifted by Prathma Blood Bank, Ahmedabad, Gujarat.

Glassware used in each procedure were soaked overnight in a chromic mixture ($K_2Cr_2O_7$ + concentrated H_2SO_4), rinsed thoroughly with double distilled water and dried in dust-free air. Whatman filter paper no. 42 was used to filter the MEM solution to separate them from the precipitated proteins and debris.

Experimental

Preparation of standard stock solution of DH

DH (25 mg) was accurately weighed and transferred to separate 25 mL volumetric flasks. Dissolved in 10 mL double distilled water, sonicated for 10 min and volume made up to mark with double distilled water to obtain standard stock solution having concentration 1000 $\mu\text{g/mL}$. A volume of 5 mL aliquot from this solution transferred to 50 mL volumetric flask and volume made up with double distilled water to obtain working standard stock solution of 100 $\mu\text{g/mL}$.

Preparation of borate buffer pH (9.6)

Boric acid (3.09 g) and potassium chloride (3.73 g) were accurately weighed and transferred to 250 mL volumetric flask. Volume was made up with distilled water. Then solution was transferred to 1000 mL beaker and pH of the solution adjusted to 9.6 by addition of 0.2 M of Sodium hydroxide solution. Then pH adjustment volume was made up 1000 mL with distilled water.

Extraction procedure

A volume of 500 μL of plasma sample was taken in 2 mL microcentrifuge tube, then 50 μL of drug solution was spiked and 500 μL of 5% TCA solution was added. It was centrifuged for 3 min at 5000 rpm, then supernatant was transferred in another 2 mL microcentrifuge tube and 100 μL of 0.1 N NaOH solution to make supernatant alkaline. To this microcentrifuge tube, 1 mL of 5 % IPA in n-Hexane solution was added and centrifuged for 3 min at 8000 rpm and upper organic phase was separated and remaining aqueous phase was again centrifuged with 1 mL 5% IPA in n-Hexane solution, and upper organic phase was separated and both organic phases were taken in 10 mL volumetric flask and evaporated to dryness. Then reconstituted with borate buffer to make up a volume of 10 mL and analyzed by spectrofluorimeter.

Spectrofluorimetric condition

From the working standard solution, 1 $\mu\text{g/mL}$ solution was prepared by appropriate dilution using diluent and scanned in the synchronous mode with a delta value 65 (medium sensitivity mode) from 220 to 680 nm. From the synchronous spectra of DH, 389 nm wavelength was selected for measurement of fluorescence intensity of DH (Figure 2).

Method validation

Linearity

For preparation of calibration curve of DH, 500 μL of blank plasma was spiked with 50 μL of drug solutions of concentration of 1 $\mu\text{g/mL}$, 5 $\mu\text{g/mL}$, 9 $\mu\text{g/mL}$, 13 $\mu\text{g/mL}$, 17 $\mu\text{g/mL}$, and 21 $\mu\text{g/mL}$ to get the final concentration of 5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 45 $\mu\text{g/mL}$, 65 $\mu\text{g/mL}$, 85 $\mu\text{g/mL}$, and 105 $\mu\text{g/mL}$, respectively. Then extraction was performed as discussed in extraction procedure and then fluorescence intensity of prepared solutions was measured at emission wavelength of 389 nm.^{14,15}

Precision

Intra-day and inter-day precision was determined by measuring the fluorescence intensity of DH 3 times within a

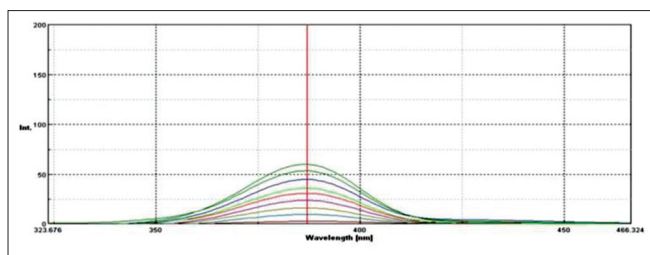


Figure 2: Overlay synchronous spectra of donepezil hydrochloride in spiked human plasma (5-105 µg/mL)

day and on three different days, respectively. For intra-day and inter-day precision, intra-day precision 25, 65, 105 µg/mL were selected. 500 µL of blank plasma was spiked with 50 µL of drug solution of concentration of 5 µg/mL, 13 µg/mL, 21 µg/mL to get the final concentration of 25 µg/mL, 65 µg/mL, and 105 µg/mL, respectively. Then extraction was performed as discussed in extraction procedure and then fluorescence intensity of prepared solutions was measured at emission wavelength of 389 nm.^{14,15}

Recovery

A volume of 500 µL of blank plasma was spiked with 50 µL of drug solutions of concentration of 1 µg/mL, 5 µg/mL, 9 µg/mL, 13 µg/mL, 17 µg/mL, and 21 µg/mL to get the final concentration of 5 µg/mL, 25 µg/mL, 45 µg/mL, 65 µg/mL, 85 µg/mL, and 105 µg/mL, respectively. Then extraction was performed as discussed in extraction procedure and then fluorescence intensity of prepared solutions was measured at emission wavelength of 389 nm.^{14,15}

Limit of detection and quantification (LOD and LOQ)

The sensitivity of the analytical method was evaluated by determining the LOD and LOQ. LOD and LOQ were calculated and reported using the following equation.

$$\text{LOD} = 3.3X \sigma/s \text{ and } \text{LOQ} = 10X \sigma/s$$

Where, σ = standard deviation of the intercept and s = slope of the calibration curve.

RESULT AND DISCUSSION

Optimization of experimental conditions

Initially, extraction of DH was carried out with the help of protein precipitation by TCA. But their synchronous spectrum was exceeding the limit of fluorescence intensity (1000 unit). Therefore, extraction of DH by only protein precipitation was not sufficient for proposed method.

Hence, extraction of DH by protein precipitation followed by liquid-liquid extraction using 5% IPA in n-Hexane as extraction solvent was carried out. After this extraction, procedure good results were obtained for DH and no interference was observed from blank for estimation of DH. In addition, it also showed good recovery of drug from plasma.

Linearity

Linearity curve shows linearity in the range of 5-105 µg/mL for DH. The correlation coefficient (R^2) was found to be 0.9965 for DH, which indicates the method is linear. Linearity study of MEM is summarized in Table 1.

Precision

Intra-day precision and inter-day precision for DH was performed by analyzing three different concentrations (µg/mL) within linearity ranges and percentage of relative standard deviation (%RSD) <10 shows that the method is precise. Results of intra-day and inter-day precision study are presented in Table 2.

Recovery

Recovery study was performed by comparing the response of the drug extracted from spiked plasma with pure drug response, and %RSD was calculated, which shows the accuracy of the method. Summary of recovery study is depicted in Table 3.

Summary of validation parameters for developed method is shown in Table 2.

Table 1: Calibration curve data of DH

Concentration (µg/mL)	Intensity	SD ^a	%RSD
5	14.20	1.21	8.52
25	20.06	1.37	6.87
45	26.56	1.41	5.32
65	34.87	1.86	5.34
85	41.15	1.72	4.19
105	46.19	1.75	3.79

DH: Donepezil hydrochloride, SD: Standard deviation, RSD: Relative standard deviation, Where, a is number of determination, n=3

Table 2: Summary of validation parameters

Parameters	DH
Detection wavelength	389 nm
Linear range (µg/mL)	5-105
Correlation coefficient R^2	0.9965
Intra-day precision %RSD ^a	≤ 10
Inter-day precision %RSD ^a	≤ 10
%Recovery	75-85
LOD (µg/ml)	4.84
LOQ (µg/ml)	14.68

DH: Donepezil hydrochloride, RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification, Where, a is number of determination, n=3

Table 3: Recovery of DH

Concentration ($\mu\text{g/mL}$)	%Recovery	%RSD ^a
5	74.83	4.16
25	85.10	4.86
45	81.57	3.76
65	81.93	2.98
85	83.33	3.67
105	79.48	3.38

DH: Donepezil hydrochloride, RSD: Relative standard deviation, Where, a is number of determination, n=3

CONCLUSION

The proposed spectrofluorimetric method for estimation of DH in human plasma found simple, precise, accurate specific, and sensitive. High recovery shows that the method is free from the interference from plasma constituents. Hence, it can be successful applied for the estimation of DH in human plasma.

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