CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

DEVELOPMENT AND VALIDATION OF NOVEL UV-SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF DONEPEZIL IN BULK AND PHARMACEUTICAL DOSAGE FORM

A. Tanuja*, CH. Srujani, Varanasi. S. N. Murthy
A. N. U. College of Pharmaceutical Sciences,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur.

Abstract:
The simple, precise and cost effective spectrophotometric methods have been developed for the estimation of Donepezil in bulk and its pharmaceutical formulations. The absorption maximum was found to be at 314nm in Zero order derivative spectrum (Method A) with the correlation coefficient 0.9988 and for the calculation of Area Under Curve (AUC)(Method B) wavelength range was found to be 304-324nm, with the correlation coefficient 0.9985. The drug follows the Beer-Lambert's law in concentration range of 4-20µg/ml for both the methods. The solvent used is methanol: water in the ratio 30:70 for the preparation of stock solution. The methods validated by analytical parameters and all the validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Donepezil in bulk and pharmaceutical dosage forms.

Key Words: *Donepezil, Area under Curve, Zero order derivative spectroscopy, Validation, Correlation Coefficient.*

Corresponding author:
A. Tanuja
A. N. U. College of Pharmaceutical Sciences,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur
E mail ID: attaluriswathi@gmail.com
Contact No: 9014299789

QR code

Please cite this article in press as A. Tanuja et al , ***Development and Validation of Novel UV-Spectrophotometric Method for the Estimation of Donepezil in Bulk and Pharmaceutical Dosage Form,*** *Indo Am. J. Pharm. Sci, 2015;2(8).*

INTRODUCTION:

Donepezil (Aricept) is a centrally acting reversible acetyl cholinesterase inhibitor. It has many therapeutic uses. It is used in the treatment of Alzheimer's disease where it is used to increase cortical acetylcholine. Donepezil is assumed to exhibit its therapeutic effect by enhancing cholinergic function. Its main action is by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase[1].

The molecular formula of Donepezil is $C_{24}H_{29}NO_3$and molecular weight is 379.49g/mol.

Donepezil is a white crystalline powder and is freely soluble in chloroform, soluble in water and in glacialacetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane[2].

By the detailed literature survey, it was found that Donepezil can be estimated by spectrophotometry (4,5,8), HPLC methods (3,6,7,9) individually or in combination with other drugs. The aim of the present work is to develop and validate new hydrotropic spectrophotometric methods for the estimation of Donepezil in bulk and pharmaceutical formulations.

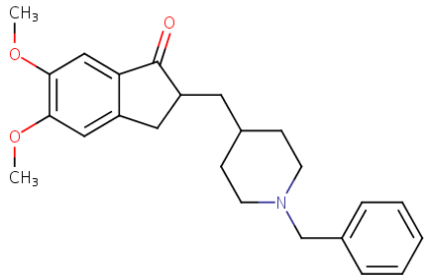

Fig 1: Structure of Donepezil

EXPERIMENTAL METHODS:

Chemicals and Reagents

Donepezil working standard was kindly provided by Mylan Laboratories, Hyderabad and was used as received. A commercial tablet formulation (Aricept 10mg) was purchased from the local market and was taken for study which contains Donepezil - 10mg. Methanol and double distilled water were used for the present study.

Instrument

A double beam UV-VIS spectrophotometer (Evolution 220, Thermo Scientific, Japan) connected to computer loaded with spectra manager software "Thermo Insight" was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Shimadzu, Japan).

Preparation of Standard Stock Solution:

The standard solution of Donepezil was prepared by dissolving accurately weighed 100 mg of the drug in 30ml of methanol and make up the volume to 100ml with double distilled water to obtain a final concentration of 1mg/mL.

Preparation of Working Standard Solution:

From the above standard stock solution, 1mL was pipetted out into a 10mL volumetric flask and the volume was made up to the mark with double distilled water to get a concentration of 100µg/mL (working standard solution).

Method A: Zero- Order Derivative Spectroscopy:

Series dilutions of the stock solution were made by pipetting out 0.4, 0.8, 1.2, 1.6 and 2.0 mL into separate 10 mL volumetric flasks and diluting to volume with double distilled water to produce the concentrations ranging from 4-20 µg /mL. The above solutions were scanned over the range of 400 nm to 200 nm against blank. The λmax was found to be at 314 nm (Figure 2,3). The present study was carried out at 314.0 nm where the Beer- Lambert's law was following properly.

The calibration curve was constructed by plotting concentration versus absorbance at 314.0 nm (Figure 4) The Statistical data is shown in Table 1.

Table 1: Statistical Data for Zero Order Derivative Spectroscopy

S. No.	Concentration (µg/mL)	Absorbance	Standard deviation*	Slope	Intercept	R^2
1	4	0.130	0.002	0.0306	0.0039	**0.9988**
2	8	0.256	0.003			
3	12	0.367	0.003			
4	16	0.481	0.002			
5	20	0.623	0.003			

***n=6**

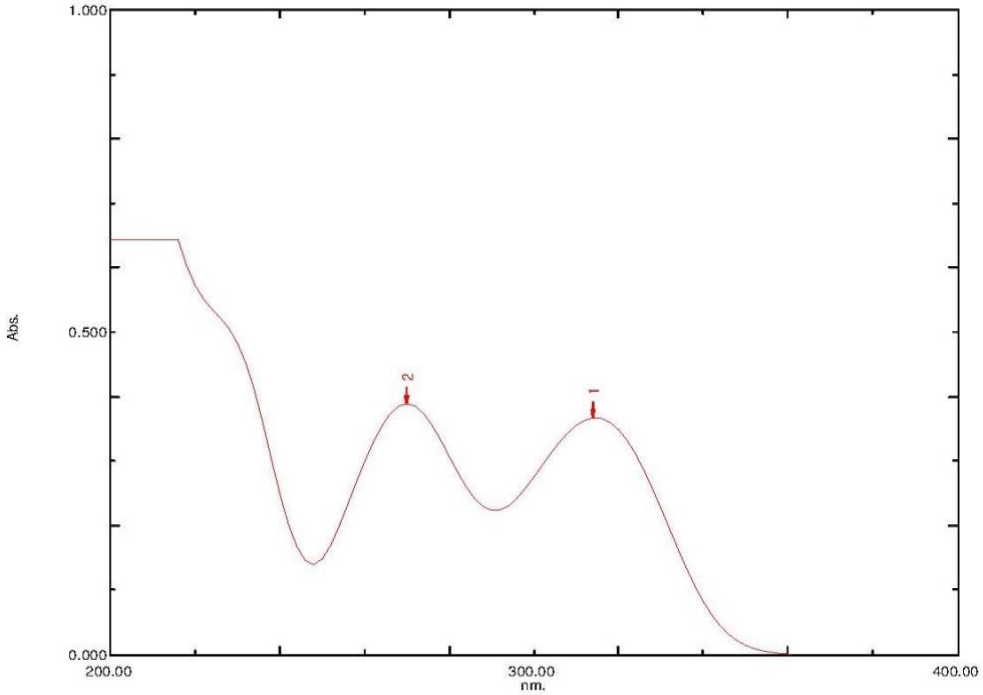

Fig 2: λ_{max} Determination for Donepezil

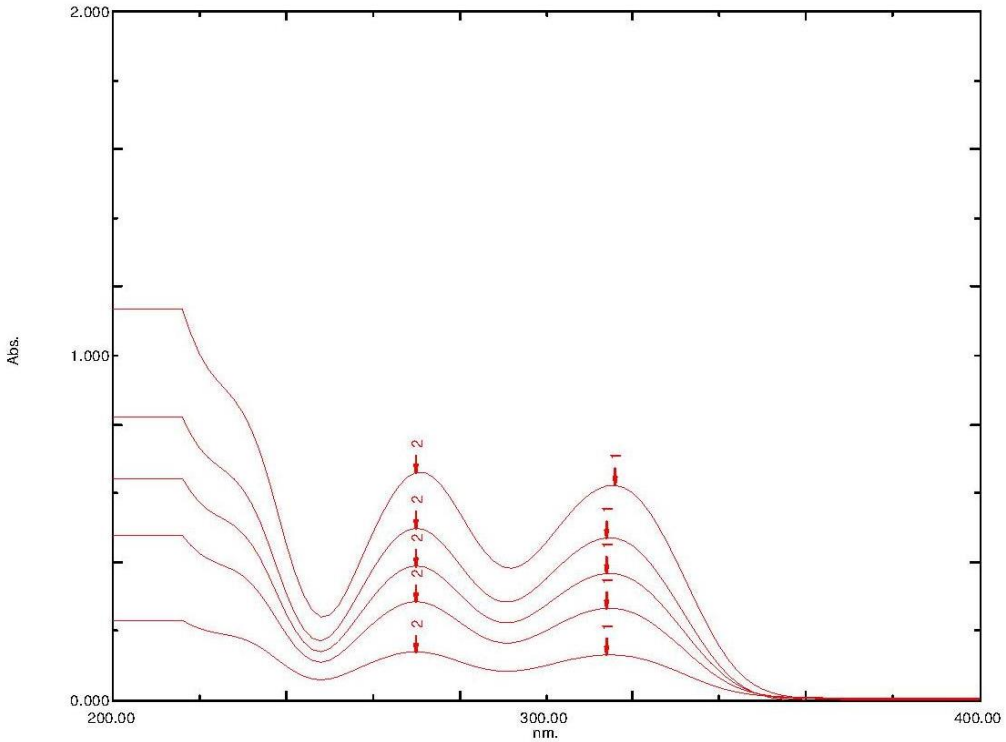

Fig 3: Linearity for Zero- Derivative Absorption Spectrum

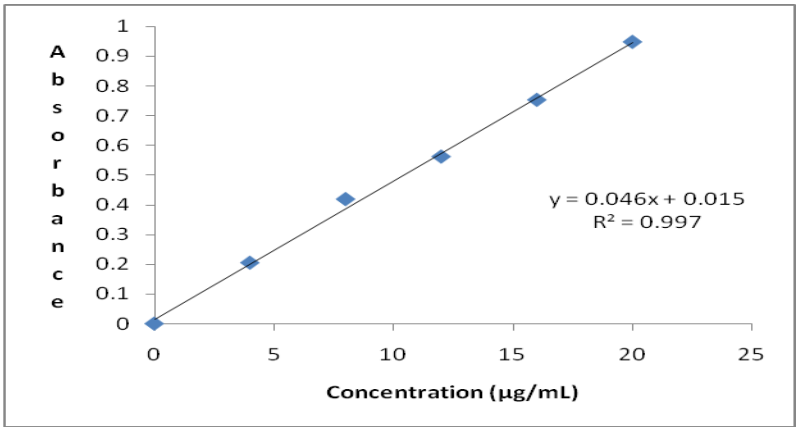

Fig 4: Calibration curve for Zero-order derivative spectrum

Method B: Area under Curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC (Figure 5). The calibration curve was constructed by plotting concentration versus AUC (Figure 6). The Statistical data is shown in Table 2.

Table 2: Statistical data for Area Under Curve

S. No	Concentration (µg/mL)	α+β	Standard deviation*	Slope	Intercept	R^2
1	4	3.8240	0.0174	0.7903	0.3376	**0.9985**
2	8	6.8122	0.0145			
3	12	9.7651	0.0248			
4	16	13.021	0.0369			
5	20	16.0184	0.0819			

* n=6

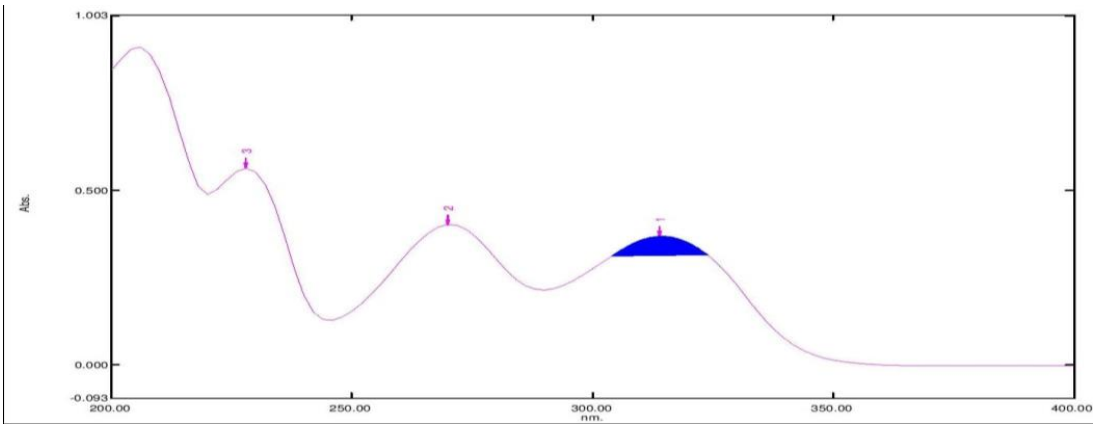

Fig 5: Area Under Curve for Donepezil

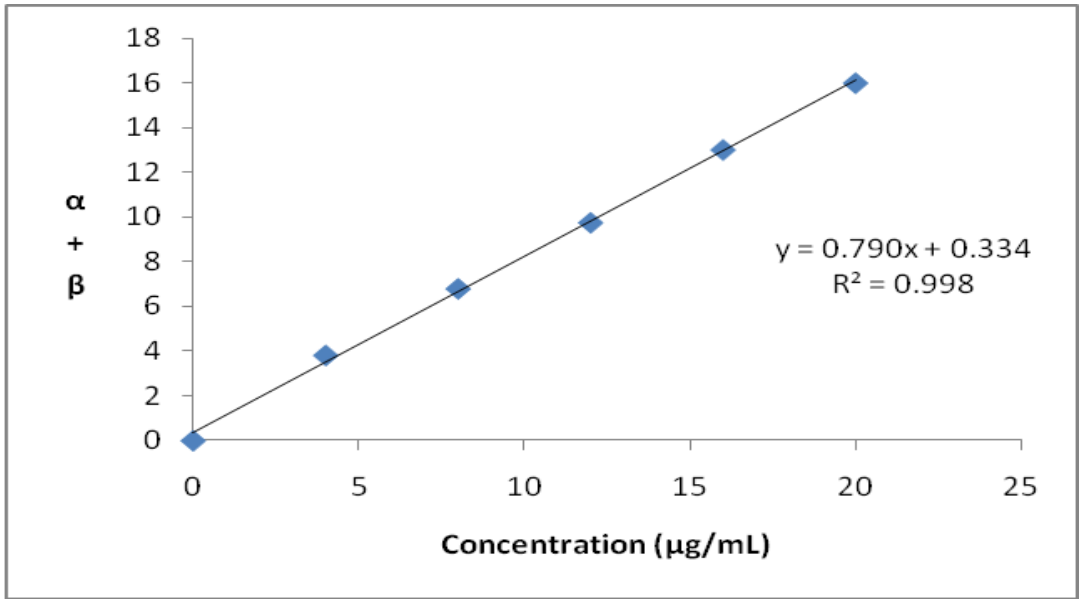

Fig 6: Calibration curve for Area under curve.

Estimation of Donepezil in Bulk & In Formulation:

For the analysis of drug in bulk, accurately weighed 100 mg sample was dissolved in 30ml of methanol initially and then volume was made up to 100 mL with double distilled water in a suitable volumetric flask. After suitable dilution with double distilled water, the spectrums of the final sample were recorded against blank as water.

For the analysis of the pharmaceutical dosage form, ten tablets of Donepezil were weighed and were finely powdered. A quantity of powder equivalent to 50 mg of the drug was transferred to a 50 mL volumetric flask and dissolved in 15ml of methanol and then with double distilled water up to the mark by keeping on an ultrasonicaton for 5 to 10 minutes. The solution was filtered through Whatmann filter paper (No. 41). From that stock solution further dilution was made with distilled water to get required concentration. The concentration of Donepezil was determined by measuring the absorbance of sample solution at 314nm. The assay procedure was repeated six times (n=6).The result of marketed formulation analysis was given in the table 3.

Table 3: Assay of the Marketed Formulation

Analysis method	Label claim (mg)	Amount found(mg)	% Recovery
A	10 mg	9.93	99.3
B	10mg	10.05	100.5
Bulk drug	100mg	99.95	99.95

Method Validation

Linearity:

For all the methods, 6-point (4.0–20.0 µg/ mL) calibration curves were prepared on 3 different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression method and the results were given in the table 4.

Precision:

The intraday and interday precisions of the proposed spectrophotometric methods were determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of Donepezil (6.0, 8.0, and 10.0 µg/mL) and the results were reported in terms relative standard deviation (RSD) given in the tables 5 & 6 respectively.

Table 4: Linearity studies of Donepezil by proposed methods

S. No.	Parameter	Method A	Method B
1	Linearity(µg/mL)	4-20	4-20
2	Slope	0.0306	0.7903
3	Intercept	0.0039	0.3376
4	Correlation coefficient	0.9988	0.9985

Table 5: Intra-day and Inter-day Precision data of Donepezil (zero order):

Concentration taken (µg/mL)	Intra-day precision		Inter-day precision	
	Mean ± SD*	% RSD	Mean ± SD*	% RSD
8	0.253±0.00132	0.521	0.257±0.00154	0.599
12	0.366±0.000553	0.151	0.367±0.00321	0.874
16	0.478±0.000316	0.066	0.485±0.00174	0.358

* n=3

Table 6: Intra-day and Inter-day Precision data of Donepezil (AUC)

Concentration taken (µg/mL)	Intra-day precision		Inter-day precision	
	Mean ± SD*	% RSD	Mean ± SD*	% RSD
8	6.789±0.0310	0.456	6.700±0.030	0.447
12	9.814±0.0444	0.452	9.258±0.0768	0.829
16	12.955±0.0376	0.290	12.842±0.1439	1.12

* n=3

Table 7: Recovery studies of Donepezil by proposed methods:

Concentration taken (µg/mL)	Spiked level (%)	Amount added (mg)	%Recovery			
			A	B	A	B
16	50	8	23.89	24.02	99.5	100.08
16	100	16	32.18	31.91	100.5	99.7
16	150	24	39.89	40.12	99.7	100.3

Accuracy:

This parameter was evaluated by the percent recovery studies at concentration levels of 50, 100 and 150% which consisted of adding known amounts of Donepezil reference materials to a pre quantified sample solution. The recovery was verified by estimation of drugs in triplicate preparations at each specified concentration level.[10] The spectrums were recorded in the UV range and then analyzed. The results are reported in terms of % recovery given in the Table 7.

DISCUSSION:

The methods discussed in the present work provided a convenient and accurate way for the analysis of Donepezil in bulk and in pharmaceutical dosage form. The absorbance maxima of Donepezil was found to be 314nm for the method A and for method C the area under curve in the range of 304-324nm was selected for the analysis. Linearity for the two methods was observed in the concentration range of 4-20 µg/mL as shown in the table 4. The assay of the three methods was found to be within the range of 98-102% as shown in the table 3. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. In both the intra-day and inter-day precision study for two methods the %RSD was found to be less than 2.0 indicating the good precision of the method as shown in the tables 5, 6 respectively. The validation of proposed methods was further confirmed by recovery studies, the %recovery values vary from 98- 102% as shown in the table 7. Based on results obtained, it was found that the proposed methods were found to be accurate, precise and reproducible and can be employed for routine quality control analysis of Donepezil in tablet dosage form.

CONCLUSION:

The proposed methods were found to be simple, sensitive, economical, accurate and precise and showed no interference from the common additives and excipients. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. Hence the proposed methods can be routinely used for the estimation of Donepezil in bulk and pharmaceutical dosage form.

ACKNOWLEDGEMENT:

The authors are thankful to Mylan Laboratories Limited, Hyderabad for providing gift sample of Donepezil and University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur for providing instrumental facilities to carry out this work.

REFERENCES:

1.http://www.drugbank.ca/drugs/db01067.
2.http://www.rxlist.com/aricept-drug.htm
3.SudheerBabu, Naga Raju K, RP-HPLC Method Development and Validation of Donepezil Hydrochloride, International Journal of Pharmacy and Pharmaceutical Sciences, 2012, 4(2).
4.ArunShirwaikar, Sarala Devi, RajaGopal P L, Kiron S S, Sreejith K R, Development and Validation of Analytical Method for Determination of Donepezil Hydrochloride in Pure and Dosage Forms, Asian Journal of Pharmaceutical and Clinical Research, 2014 7(1).
5.Sangshetti J N, Mahaparale P R, Paramane S and Shinde D B, Spectrophotometric Estimation of Donepezil Hydrochloride in Bulk and Tablet Formulation, Science Alert, 2008,3(1): 109-112.
6.Abbas SS, Fayez YM, Abdel Fattah Leh-S, Stability Indicating Methods for Determination of Donepezil Hydrochloride According to ICH guidelines, PubMed, 2006; 54(10):1447-50.
7.Yasui Furukori N, Furuya R, Takahata T, Tateishi T, Determination of Donepezil, an Acetylcholinesterase Inhibitor, in Human Plasma by High Performance Liquid Chromatography with Ultraviolet Absorbance Detection, J Chromatogr B Analyt Technol Biomed Life Sci. 2002 Mar 5;768(2):261-5.
8.RajgorVM, Parmar PT, Patel CN, Patel AS, Analytical Method Development and Validation for the Simultaneous Estimation of MemantineHCl and Donepezil HCl in Bulk and Pharmaceutical Dosage Form, International Journal of Pharmaceutical Research and Bio-Science,2014;3(3): 188-197.
9.S. Dharmaraj Santhosam, S. Kannan, S. Lakshmi Devi, Development and Validation of RP-HPLC Method for Estimation of Donepezil HCl from Bulk and Marketed Dosage Forms, 2010;2(6):62-67.
10. Varanasi.S.N.Murthy, Ch.Srujani, A.Tanuja, D.Krishna Sowmya ,Development and Validation of Novel UV- Spectrophotometric Method for the Estimation of Bosentan in Bulk and Pharmaceutical Dosage Form, Inventi Rapid: Pharm Analysis & Quality Assurance, 2015;(1):1-6.