IJBPAS, December, 2023, 12(12): 5599-5611

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SPECTROPHOTOMETRIC ANALYSIS OF DONEPEZIL BY MULTIVARIATE CALIBRATION ANALYSIS

VIGNESH K, KOKILAMBIGAI K. S* AND MANIKANDAN K

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Chengalpattu District, Tamil Nadu, India

*Corresponding Author: Dr. Kokilambigai K S; E Mail: kokilams@srmist.edu.in; kokilampharm@gmail.com

Received 19th Oct. 2022; Revised 16th Nov. 2022; Accepted 1st April 2023; Available online 1st Dec. 2023

https://doi.org/10.31032/IJBPAS/2023/12.12.7620

ABSTRACT

The aim of this research work was to develop a simple, accurate, sensitive and validated Ultra Violet (UV) spectrophotometric assay using multivariate regression method for the analysis of Donepezil. This multivariate calibration technique was based on equations constructed using linear regression analysis using the correlation between absorbance and concentration at five selected equidistant wavelengths. Donepezil had a maximum wavelength of 220 nm and 263 nm. The findings were statistically analysed for significance. A linear plot in the concentration range of 7-13 µg/mL, with a regression co-efficient of 0.999 was obtained. The % RSD for intra-day and inter-day precision were 0.3162, 0.3145 and 1.0416, 0.6892 respectively. The assay was determined and found to be 98.13% - 101.50 % w/w.

Keywords: Donepezil, Anti Alzheimer drug, UV spectrophotometry, Multivariate calibration, Assay, ICH guidelines

INTRODUCTION:

The novel anti-medication for Alzheimer's is Donepezil Hydrochloride (DNE) **(Figure. 1)** which effectively inhibits acetylcholine esterase and its IUPAC name was found to be 2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one;hydrochloride **[1]**. The chemical formula is $C_{24}H_{29}NO_3$, HCl was discovered to have a molecular weight of 379.5 g mol-1**[2]**. It is listed in the Indian Pharmacopoeia **[3]**. Donepezil is a selective inhibitor of acetyl cholinesterase in CNS than in the

periphery by butyryl cholinesterase, according to in vitro studies **[4]**. Clinical trials have shown that Donepezil is safe and does not have the hepatotoxicity risks associated with tacrine which is an acridine-based inhibitor of cholinesterase **[5]**. This drug has linear and dose-proportional pharmacokinetics where plasma clearance is slow and a half-life of 70-80 h which is long, according to studies performed during phase 1 in the United States **[6]**. The cytochrome P-450 isoenzyme CYP-3A4, with some assistance from CYP2D6, is the primary source for the process of Donepezil **[7]**. For nearly a half-century, theophylline, a methyl xanthine alkaloid, has been used to treat patients with reversible airway obstruction those are suffering with asthma and those with inflamed bronchi **[8]**. Several analytical methods are involved in quantification of Donepezil which includes UV **[9]**, HPLC **[10-11]**, LC-MS/MS [12-13], UPLC **[14]** and HPTLC **[15]**.

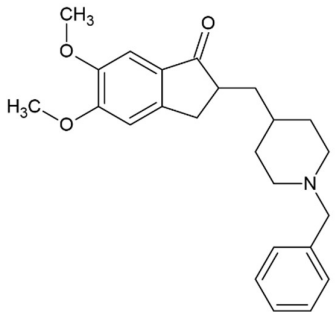


Figure 1: Chemical strucutre of Donepezil

The suggested technique provides higher confidence in results as it directly evaluates DNE and has been attested with greater accuracy and precision than a classical UV-Visible assay. This technique is more cost-effective, direct, and rapid than other methods and can be used for bulk drugs and various dosage forms. This multivariate standardization method simplifies the individual result and converts it into an "m" value as a reliant variable. Within optimized conditions, this analytical technique would provide excellent sensitivity, resolving power, expeditiousness, and cost-effectiveness for a validated quantification of DNE. The absorbance of an analyte (X), i.e., DNE, is scanned at 10 different absorbances (λ = 216, 218, 220, 222, 224 nm and 259, 261, 263, 265, 267 nm); the following formula can then be applied for any preferred wavelength **[16-23]**.

$A_{\lambda 1} = a\ X\ C_x + \ k_1$ ---------- (1)

$A_{\lambda 2} = b\ X\ C_x + \ k_2$ ---------- (2)

$A_{\lambda 3} = c\ X\ C_x + \ k_3$---------- (3)

$A_{\lambda 4} = d\ X\ C_x + \ k_4$---------- (4)

$A_{\lambda 5} = e\ X\ C_x + \ k_5$---------- (5)

Where A_λ is the analyte's absorbance, a, b, c, d, and e being slopes of the analyte's linear regression functions; intercepts are denoted as k1, k2, k3, k4, k5 at the each five specified wavelengths, and Cx is the analyte's concentration. The selected five equation systems (1–5) listed above can be summarised as follows:

$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + \ d\ X\ C_x + \ e\ X\ C_x + \ K_T$---------- (6)

The above equation can be further condensed to

$A_T = C_x\ (a + b + c + d + e) + \ K_T$---------- (7)

A_T and K_T are the summations of the absorbance acquired cum totality of intercepts of regression equations at selected each five wavelengths, respectively. The following formula computes the concentration of the analyte X.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

MATERIALS AND METHODS

Chemicals and reagents

- Water
- DNE was obtained as a gift sample from Ideal Analytical and Research Institute, Pondicherry. The marketed tablet formulation used was Aricep Eisai pharmaceuticals, India (Label claim – 10 milligram DNE), acquired from a local market.

Instrumentation

- LAB INDIA 3092 UV-Visible double beam spectrophotometer
- Ultra Sonicator Bath
- Analytical balance
- Micropipette

Analytical method development

Choice of the solvent

In Water, DNE was found to be freely soluble. Hence, Water was used for further dilutions of both standard and sample.

Standard stock solution

DNE standard stock solution was prepared by the dissolution of 10 mg of the standard drug in 10 mL of Water and then making up to the mark in a 100 mL standard flask with the same solvent. 1 mL of this solution was transferred to another 10 mL volumetric flask and made up to 10 mL with diluent to obtain a 10 µg/mL concentration. Several concentrations (7-13 µg/mL) of solution were prepared from this standard stock solution.

Determination of λmax

The standard stock solution was diluted in water to obtain 20 µg/mL. These solutions were measured in the Ultra-Violet region from 200 - 400 nm. The λ_{max} was obtained as 220 nm and 263 nm **(Figure 2)**. The linear curve was obtained with a graph plotting the absorbance against the concentration **(Table 1, 2)**. The solutions were scanned across the range surrounding 220 nm, i.e., 216, 218, 220, 222, 224 nm and 263 nm, i.e., 259, 261,

263, 265, 267 nm to enhance the correlation and diminish instrumental oscillations.

Preparation of sample solution

Twenty tablets of DNE were accurately weighed and powdered. A weight corresponding to 10 mg was measured into a 10 ml volumetric flask, dissolved, and made up to the mark with Water to obtain 100 µg/mL. This solution was then filtered and used for further analysis.

Method Validation

According to ICH Q2B guidelines this method was validated for sensitivity, precision, accuracy, and linearity **[24]**.

Linearity

The different concentrations over the 7-13 µg/mL range were prepared from the standard stock solution of DNE. To minimize instrumental fluctuations and to better the correlation, these solutions were scanned over a range of wavelengths surrounding their absorbance maxima at 216, 218, 220, 222, 224 nm and 259, 261, 263, 265, 267 nm respectively. The absorbances were recorded, and the standardizations were obtained by plotting a concentration vs. absorbance graph **(Figure 3, Table 1 & 2)**.

Table 1: UV Calibration data at five distinct wavelengths (λ_{max} - 220 nm)

Concentration (µg/mL)	Absorbance#				
	216 nm	218 nm	220 nm	222 nm	224 nm
7	0.306	0.315	0.324	0.315	0.303
8	0.364	0.378	0.392	0.376	0.362
9	0.431	0.446	0.461	0.446	0.423
10	0.489	0.513	0.531	0.511	0.482
11	0.552	0.578	0.593	0.583	0.542
12	0.612	0.641	0.662	0.648	0.605
13	0.672	0.704	0.727	0.714	0.659

#Average of 5 determinations; UV= Ultra violet

Table 2: UV Calibration data at five distinct wavelengths (λ_{max} - 263 nm)

Concentration (µg/mL)	Absorbance#				
	259 nm	261 nm	263 nm	265 nm	267 nm
7	0.139	0.142	0.144	0.137	0.139
8	0.168	0.174	0.175	0.170	0.167
9	0.195	0.202	0.206	0.203	0.195
10	0.225	0.231	0.238	0.231	0.224
11	0.256	0.264	0.269	0.266	0.254
12	0.291	0.294	0.301	0.296	0.287
13	0.319	0.320	0.326	0.325	0.312

#Average of 5 determinations; UV= Ultra violet

The sensitivity of the method was determined by calculating the detection and quantification limit using the below formula.

$LOD = 3.3\ \sigma/S$ …….... (8)

$LOQ = 10\ \sigma\ /S$..... (9)

Here, σ is the standard deviation (SD) of the lowermost concentration and

S is the slope of the standard curve.

Precision

To assess the intra-day and inter-day precision, 20 µg/mL solution was scanned six times in a short interval on one day for

intraday precision and six different days for inter-day precision.

Accuracy

The recovery studies for the suggested technique were resolved at 80%, 100%, and 120% using the standard addition technique. The standard and sample stock solutions were prepared. 1.2 mL of the standard was pipetted into three 10 mL volumetric flasks. 0.8, 1.0 and 1.2 of sample solution were added, respectively, making up to a capacity of 10 mL with water. These solutions were measured with a UV spectrophotometer, and the percentage recovery was calculated.

Assay

The amount of DNE present in the tablet formulation was calculated by measuring the absorbance of the extracted tablet solution at 220 and 263 nm.

RESULTS AND DISCUSSION

The λ_{max} of DNE was found to be 220 nm and 263 nm with Water as the solvent as shown in **Figure 2**.

The technique is linear within the assigned concentration range of 7-13 µg/mL. The linear regression analysis shows an excellent linear relationship with R^2= 0.9991 - 0.9998 for all the calibration plots. The % relative standard deviation for precision was found to be 0.3162, 0.3145 and 1.0416, 0.6892. The LOD and LOQ obtained are 0.0072 µg/mL, 0.0218 µg/mL and 0.0066 µg/mL, 0.0201 µg/mL respectively. Therefore, the values fell according to ICH guideline limits of validation parameters.

Linearity

The linearity was recorded at 216, 218, 220, 222, 224 nm and 259, 261, 263, 265, 267 nm in the concentration range of 7-13 µg/mL and depicted in **Figure 3**, and corresponding calibration curves are presented in **Figures 4 to 13**. For each wavelength, the low values of % relative standard deviation show that the technique is accurate and precise. The LOD and LOQ were calculated and reported in **Table 3, 4**.

Precision

The low standard deviation values indicate that this technique is specific, and % RSD for the intra-day and inter-day precision were found to be 0.3162 and 0.3145, respectively. It lies within the limits of less than 2% at each wavelength. The low percentage value of relative standard deviation reveals that the suggested technique is accurate and precise **(Figure 14, 15)**.

Recovery

As per ICH guidelines, the % recovery of DNE was found to be from 99.00 – 101.33% w/w. The recovery was within the acceptable range of 97 - 103 % w/w **(Figure 16, Table 5, 6)**.

Assay:

The UV absorbance of the tablet formulation was recorded at 220 and 263 nm. The quantity and assay percentages are 9.96 and 9.92 mg and 99.63 % and 99.17% w/w, respectively, with % RSD values as in **Table 7**.

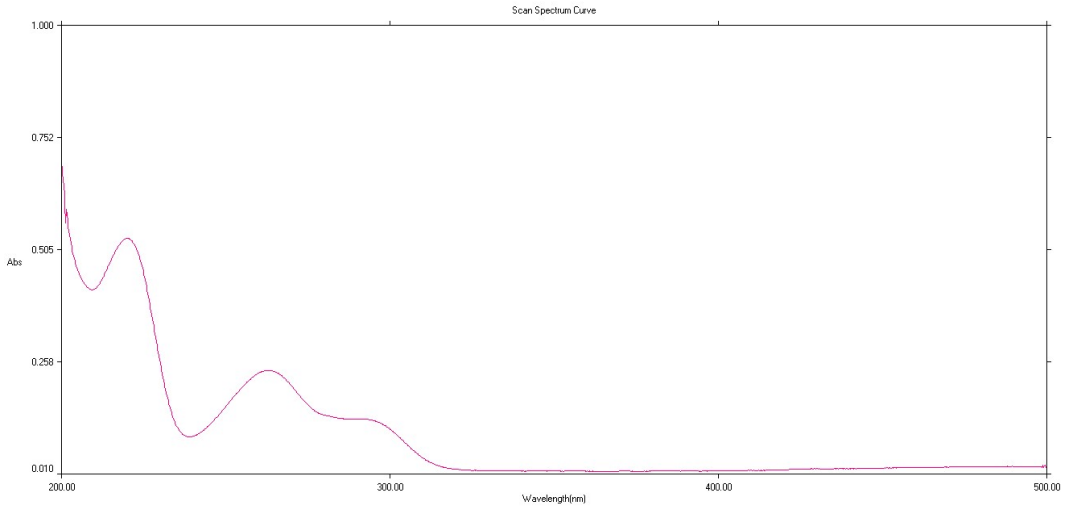


Figure 2: UV spectrum of Donepezil (20 µg/mL), λ_{max} at 220 nm & 263 nm

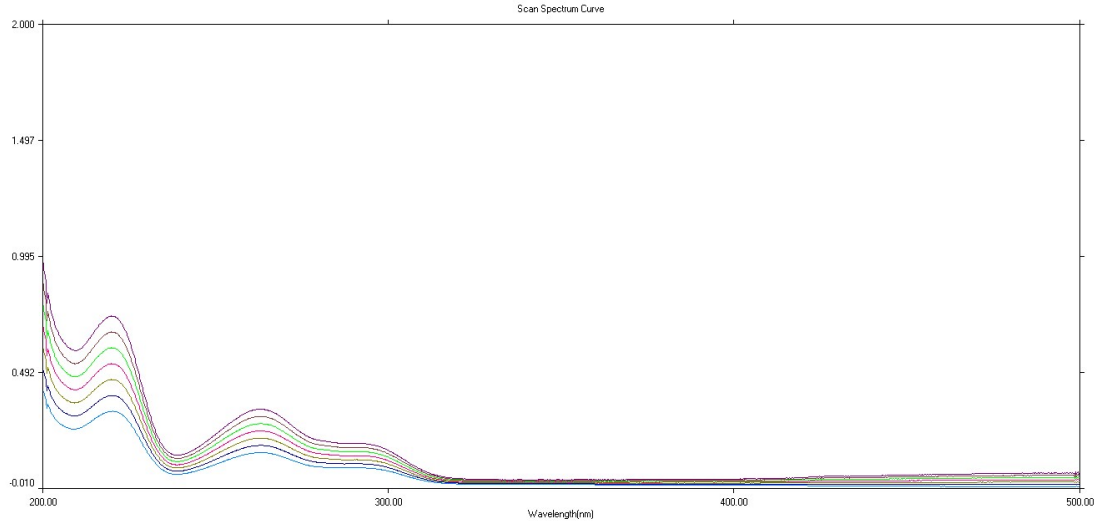
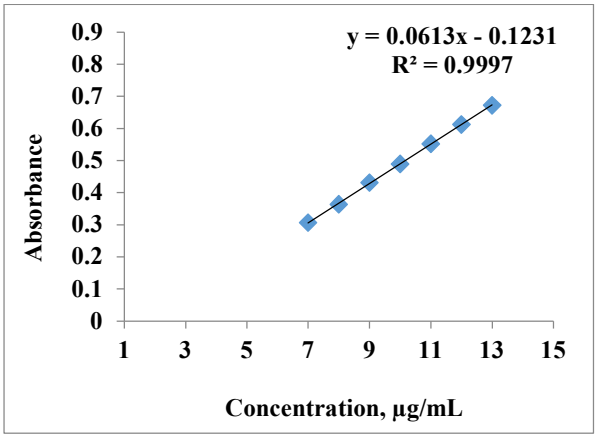


Figure 3: UV Spectrum of Donepezil showing linearity at 220 and 263 nm



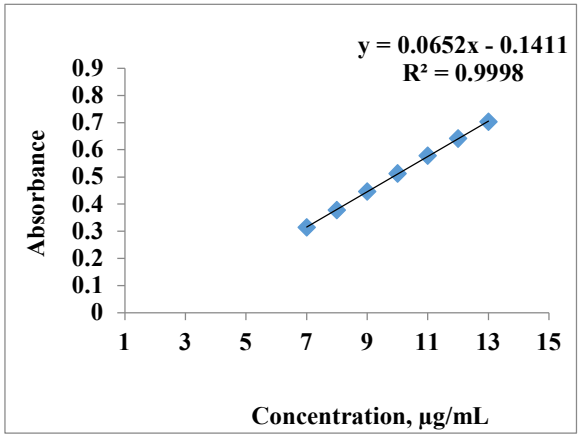


Figure 4: Calibration curve at 216 nm **Figure 5: Calibration curve at 218 nm**

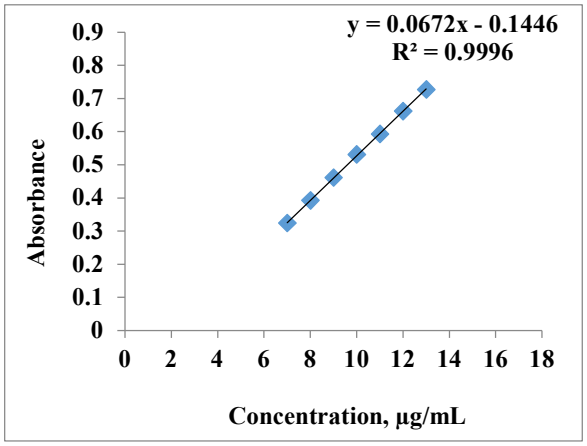


Figure 6: Calibration curve at 220 nm

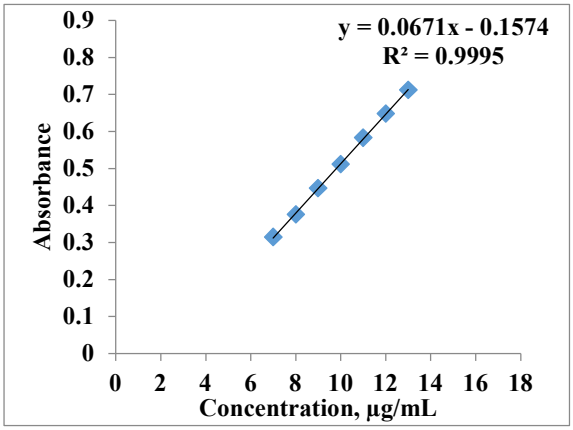


Figure 7: Calibration curve at 222 nm

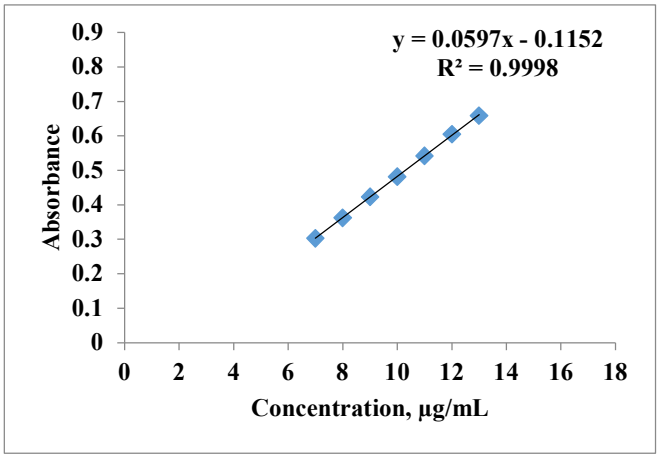


Figure 8: Calibration curve at 224 nm

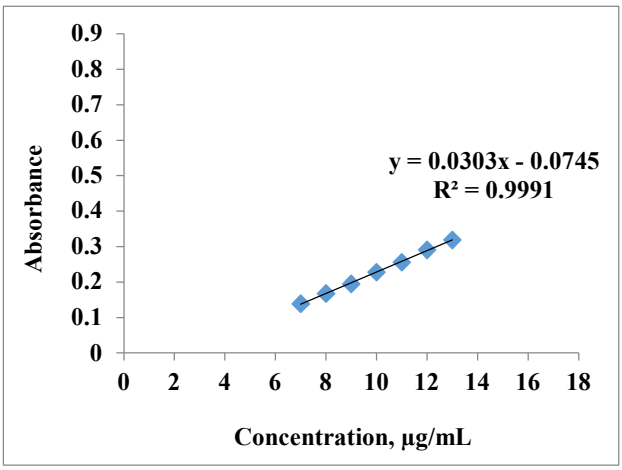


Figure 9: Calibration curve at 259 nm

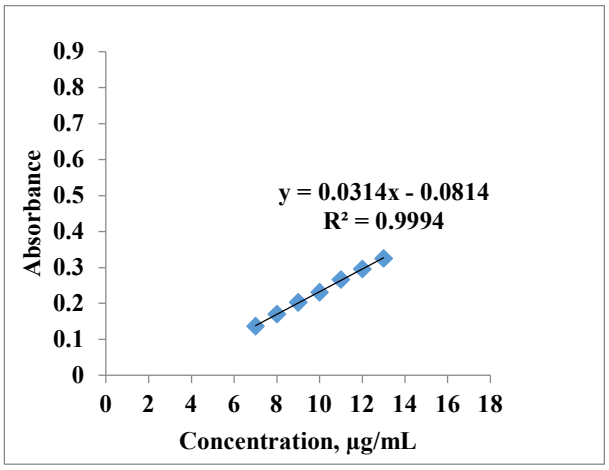


Figure 10: Calibration curve at 261 nm

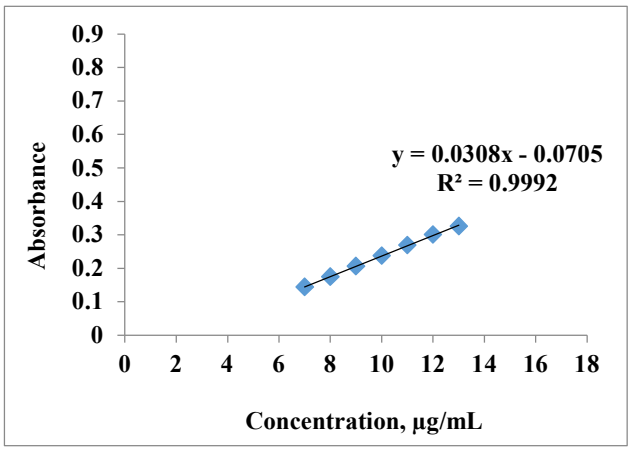


Figure 11: Calibration curve at 263 nm

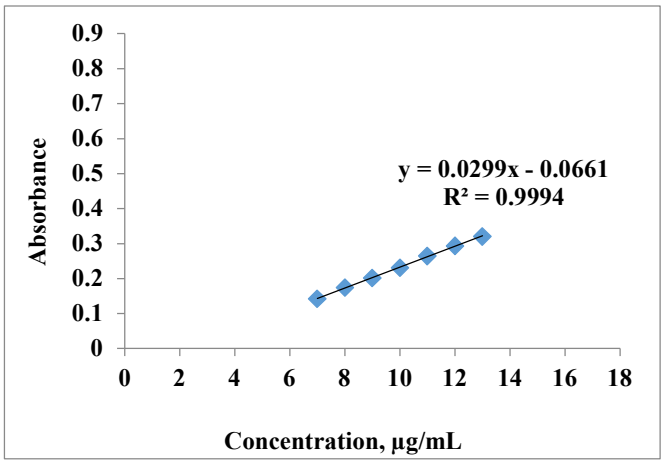


Figure 12: Calibration curve at 265 nm

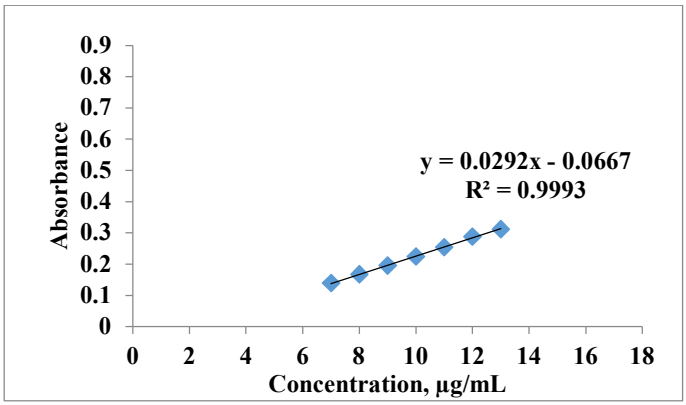


Figure 13: Calibration curve at 267 nm

Table 3: Linearity data with LOD and LOQ at selected five wavelengths λ_{max} at 220 nm

Wavelength (nm)	Regression equation	R^2	LOD (µg/mL)	LOQ (µg/mL)	% RSD
216	y = 0.0613x - 0.1231	0.9997	0.0065	1.0199	0.4082
218	y = 0.0652x - 0.1411	0.9998	0.0066	0.0202	0.3968
220	y = 0.06172x - 0.1446	0.9996	0.0072	0.0218	0.4153
222	y = 0.0671x - 0.1574	0.9995	0.0087	0.0266	0.5188
224	y = 0.0597x - 0.1152	0.9998	0.0062	0.0188	0.3898

*nm = Nano meter; µg/mL = Microgram per millilitre

Table 4: Linearity data with LOD and LOQ at selected five wavelengths λ_{max} at 263 nm

Wavelength (nm)	Regression equation	R^2	LOD (µg/mL)	LOQ (µg/mL)	% RSD
259	y = 0.0303x - 0.0745	0.9991	0.0069	0.0210	0.9252
261	y = 0.0299x - 0.0661	0.9994	0.0057	0.0174	0.7487
263	y = 0.0308x - 0.0705	0.9992	0.0066	0.0201	0.8491
265	y = 0.0314x - 0.0814	0.9994	0.0057	0.0175	0.7544
267	y = 0.0292x - 0.0667	0.9993	0.0059	0.0181	0.8040

*nm = Nano meter; µg/mL = Microgram per millilitre

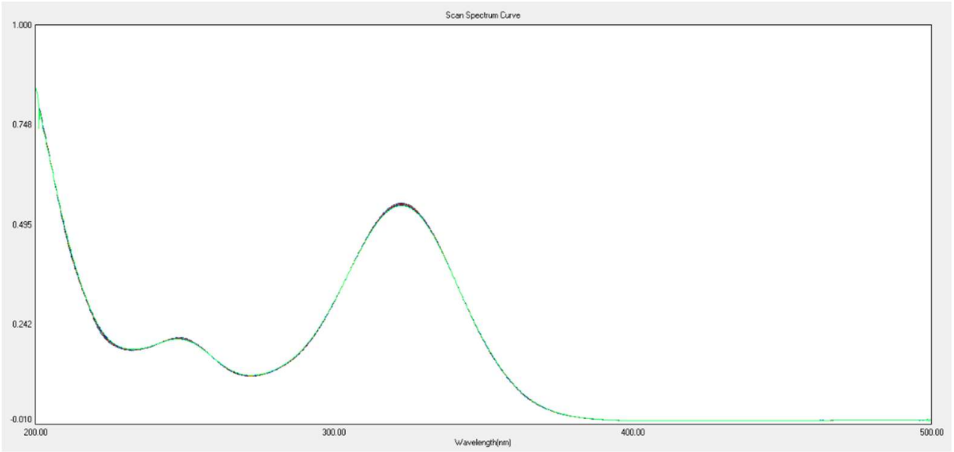


Figure 14: UV spectra showing intraday precision

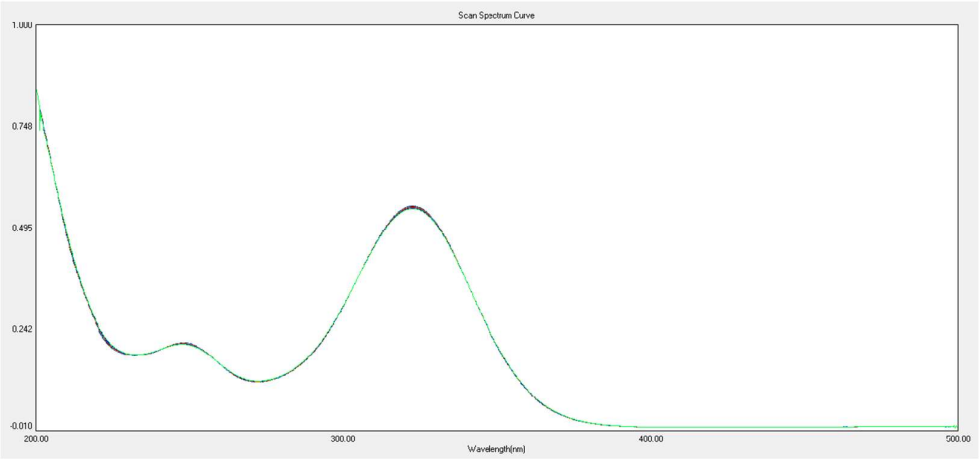


Figure 15: UV spectra showing interday precision

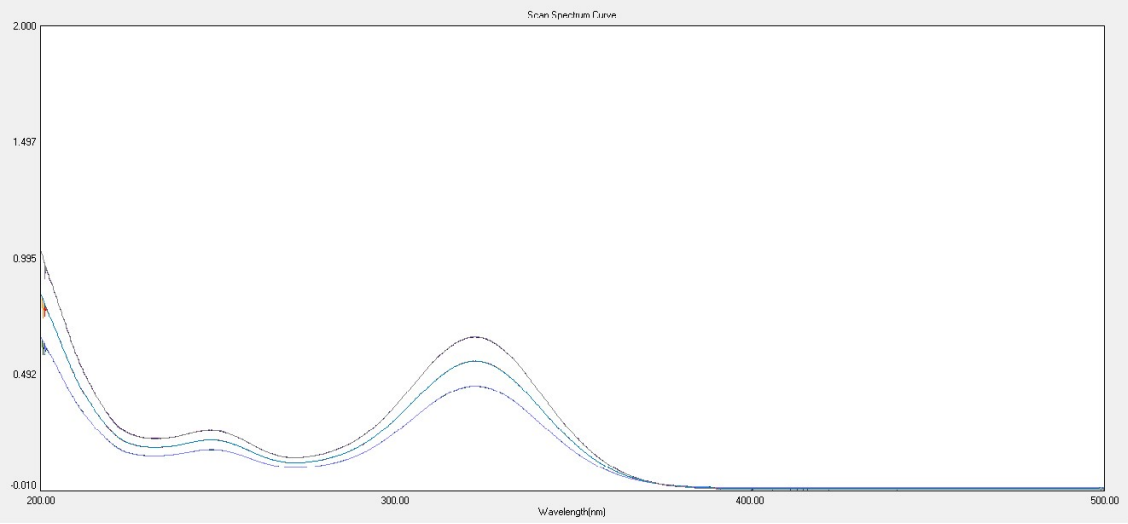


Figure 16: UV Spectrum showing accuracy of Donepezil

Table 5: Recovery Studies (λ_{max} at 220 nm)

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
216 nm	2	8	0.371	9.9	99.00
		13	0.451	15.1	100.67
		18	0.532	19.8	99.00
218 nm	2	8	0.421	10	100.00
		13	0.509	14.9	99.33
		18	0.601	20	100.00
220 nm	2	8	0.448	10.1	101.00
		13	0.545	15	100.00
		18	0.635	20.1	100.50
222 nm	2	8	0.432	9.9	99.00
		13	0.522	15.2	101.33
		18	0.614	19.9	99.50
224 nm	2	8	0.371	10.1	101.00
		13	0.441	14.9	99.33
		18	0.512	20.2	101.00

Table 6: Recovery Studies (λ_{max} at 263 nm)

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
259 nm	4	4	0.371	7.9	98.75
		6	0.451	10.1	101.00
		8	0.532	12.2	101.67
261 nm	4	4	0.421	8.1	101.25
		6	0.509	9.85	98.50
		8	0.601	11.9	99.17
263 nm	4	4	0.448	8.12	101.50
		6	0.545	9.9	99.00
		8	0.635	12.2	101.67
265 nm	4	4	0.432	8	100.00
		6	0.522	10.03	100.30
		8	0.614	12.07	100.58
267 nm	4	4	0.371	8.05	100.63
		6	0.441	10.1	101.00
		8	0.512	12.1	100.83

Table 7: Assay of Donepezil

Label claim (mg)	λ_{max} - 220 nm		λ_{max} - 263 nm	
	Amount obtained (mg)	% Assay	Amount obtained (mg)	% Assay
10	9.95	99.50	9.83	98.30
10	9.96	99.60	9.95	99.50
10	9.98	99.80	9.97	99.70
Average	9.96	99.63	9.92	99.17
SD		0.1528		0.7572
% RSD		0.1533		0.7636

CONCLUSION:

This novel multivariate technique is more accurate, precise, reproducible, cost-effective, and sensitive than classical UV-Visible Spectrophotometry for Donepezil assay. This multilinear regression analysis is proven desirable for the testing standard drug and other dosage forms of DNE. This method is validated using ICH Quality Guidelines and found to be within the set limits of validation. This is a simple working procedure compared to expensive and intricate techniques such as HPLC and HPTLC, and hence can be employed for

routine analysis of DNE formulations in bulk drugs and pharmaceuticals.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects

ACKNOWLEDGMENTS

Authors are thankful to the Chancellor, SRM Institute of Science and Technology, and the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur for providing various reprographic sources for carrying out this work.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

FUNDING SOURCES

There is no funding to report.

REFRENCES

[1] Martindale, The Complete Drug Reference, 37th Edition.

[2] O Neil MJ, Heckelman P, Koch C, Roman K, 2006. The Merck Index, 15th Edition, 625.

[3] The Indian Pharmacopoeia, Government of India, Ministry of Health, vol/II: 2018

[4] Rogers SL, Yamanishi Y, Yamatsu K. E2020-The pharmacology of a piperidine cholinesterase inhibitor. Cholinergic Basis for Alzheimer Therapy. Boston, Birkhauser, 1991. 113th chapter, 314–320.

[5] Sherman K, Pharmacodynamics of oral E2020 and tacrine in humans. Novel approaches. Cholinergic Basis for Alzheimer Therapy. Boston, Birkhauser, 1991. 99th chapter, 321–328.

[6] Rogers SL, Cooper NM, Sukovaty R, Pederson JE, Lee JN, Friedhoff LT. Pharmacokinetic and pharmacodynamic profile of Donepezil HCl following multiple oral doses. Br J Clin Pharmacol 1998; 46(1): 7–12.

[7] Laurence LB, John SL, Keith LP. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th Edition, McGRAW HILL, New York, 2006; 659-682.

[8] Griffin JP, D Arcy PF, Speirs CJ. Manual of Adverse Drug Interactions.4th Edition, 1988;427–439.

[9] Sangshetti JN, Mahaparale PR, Paramane S, Shinde DB. Spectrophotometric Estimation of Donepezil Hydrochloride in Bulk and Tablet Formulation. Trends in Appl Sci Res 2008; 3: 109-112.

[10] Tushar GB, Patel PK. RP-HPLC method for the estimation of Donepezil hydrochloride dosage form. E-J of Chem 2009; 6(2): 594-600.

[11] Sastrya BS, Gananadhamu S, Devadasu C, Balaji K. New RP-HPLC method for estimation of Donepezil hydrochloride in pharmaceutical formulations. Int. J. Chem. Sci 2008; 6(4): 1976-1983.

[12] Shah HJ, Kundlik ML, Pandya A, Prajapati S, Subbaiah G, Patel CN. A rapid and specific approach for direct measurement of Donepezil concentration in human plasma by LC-MS/MS employing solid-phase extraction. Biomed Chromatogr 2009; 23: 141–151. doi: 10.1002/bmc.1095.

[13] Pilli NR, Inamadugu JK, Kondreddy N, Karra VK, Damaramadugu R, Rao JV. A rapid and sensitive LC-MS/MS method for quantification of Donepezil and its active metabolite, 6-o-desmethyl donepezil in human plasma and its pharmacokinetic application. Biomed Chromatogr. 2011; 25: 943–951. doi: 10.1002/bmc.1552.

[14] Mahalingam V, Vijayabaskar S, Kalaivani RA, Somanathan T. Analytical method development and validation for the analysis of Donepezil hydrochloride and its related substances using Ultra Performance Liquid Chromatography. Res J. Pharm. Tech 2017;10(8):2743-2749. doi: 10.5958/0974-360X.2017.00487.5.

[15] Jagadeeswaran M, Gopal N, Gandhimathi M, Rajavel R, Ganesh M, Sivakumar AT. Validated HPTLC method for the estimation of Donepezil HCl in bulk and its tablet dosage form. Eurasian J Anal Chem 2011;6(1):40-45.

[16] Kokilambigai KS, Seetharaman R, Kavitha J, Sowndaravel P, Lakshmi KS. Multivariate Calibration technique for the spectrophotometric quantification of Zaleplon in bulk and pharmaceutical formulations. J. Pharm. Sci. Res 2017;9(6):,824-9.

[17] SaiSusmitha A, Kokilambigai KS, Lakshmi KS. Spectrophotometric quantification of Telmisartan employing multivariate calibration technique in bulk and pharmaceutical formulations. Res. J. Pharm. Technol 2019;12(4):1799-805. DOI: 10.5958/0974-360X.2019.00300.7.

[18] Madhan S, Kavitha J, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of Ivermectin in Pharmaceutical Formulation. Asian

J. Pharm. Clin. Res. 2019;12(2): 444-51.

[19] Durgadevi P, Kokilambigai KS, Lakshmi KS. First order derivative spectrophotometric method for the quantification of Telmisartan employing Multivariate Calibration Technique. Res. J. Pharm. Technol 2020;13(2):774-80. DOI: 10.5958/0974-360X.2020.00146.8.

[20] Naveena Rani D, Kokilambigai KS, Lakshmi KS. Multivariate calibration technique for the spectrophotometric quantification of Rasagiline in bulk drug and pharmaceutical formulation. Res. J. Pharm. Technol 2020;13(2):843-9. DOI: 10.5958/0974-360X.2020.00159.6.

[21] Mani Aravinth R, Kokilambigai KS, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi KS. Spectrophotometric quantification of Granisetron in bulk drug and pharmaceutical formulations employing multivariate calibration technique. J. Med. Pharm. Allied Sci 2021;10(4):3435-9. DOI: 10.22270/jmpas. V10I4.1442.

[22] Vimal Ravi, Kokilambigai KS, Kavitha J, Seetharaman R, Lakshmi KS. Multivariate UV Spectrophotometric Quantification of Cilnidipine in Bulk Drug and Pharmaceutical Formulations. J. Med. Pharm. Allied Sci 2022;11(2):4672-8.

[23] Kokilambigai KS, Logesh M, Anusha Reddy C, Seetharaman R, Kavitha J, Lakshmi KS. Multivariate Calibration Technique for Spectrophotometric Quantification of Ondansetron in Bulk Drug and Pharmaceutical Formulations. J. Med. Pharm. Allied Sci 2022;11(4):4777-82.

[24] ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. 2005 Geneva, Switzerland 2005.