CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.17019432

https://www.iajps.com/volumes/volume12-august-2025/58-issue-08-august-25/

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

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RIVASTIGMINE IN PHARMACEUTICAL DOSAGE FORM BY USING UV-VISIBLE SPECTROSCOPY

P.T. Nagaraju*, Y.Sai Pravallika, S.Amreen Sulthana,T.Mayuri
Dr. K. V. Subba Reddy Institute of Pharmacy,
Dupadu, Kurnool, A.P – 518218

Abstract:
A simple, specific, accurate and precise UV spectrophotometric method was developed and validated for the estimation of Rivastigmine in pharmaceutical dosage form. The stock solution was prepared by weighing 100mg of standard Rivastigmine in 1000ml of 0.1M HCl in volumetric flask. The final stock solution was made to produce 1g/µl with water. Further dilution was prepared as per procedure and were scanned at 209nm. The linearity was found in the concentration range of 1-6 µg/ml. the correlation coefficient was 0.9975. The regression equation was found to be Y= 0.0105X +0.2417 Recovery of Rivastigmine was found to be in the range of 98-102%. The method was validated for limit of detection limit of quantification for estimation of Rivastigmine was found to be µg/ml respectively. The Proposed method can be successfully applied for the quantitative determination of Rivastigmine in pharmaceutical dosage form.

Corresponding author:
P.T. Nagaraju,
Dr. K. V. Subba Reddy Institute of Pharmacy,
Dupadu, Kurnool, A.P – 518218
Mail I.D: nraju04@gmail.com
Contact: 9885755749

QR code

Please cite this article in** press **P.T. Nagaraju et al., Analytical Method Development And Validation Of Rivastigmine In Pharmaceutical Dosage Form By Using Uv-Visible Spectroscopy., Indo Am. J. P. Sci, 2025; 12(08).

INTRODUCTION:

Pharmaceutical Analysis [1, 2]is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative chemical analysis of the substances present in bulk and pharmaceutical preparations.

UV-Visible Spectroscopy[3]:

The prescribed unit used in UV-Visible Spectroscopy is wavelength expressed as nanometre (NM). The position of maximum Absorbance of a peak is designated as λ_{max}. The wavelength in these regions is divided into two ranges i.e. 200-400 nm (UV region) and about 400-800 nm (Visible region).

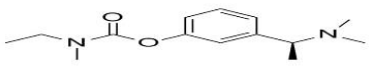
AIM AND OBJECTIVE-

The specific aim of the research:

To develop and validate UV-VIS spectroscopic method for the estimation of Rivastigmine Pharmaceutical dosage form. The developed method can successfully be applied to estimate the amount of Rivastigmine in dosage form. The proposed method was validated as per ICH and USP guidelines.

DRUG PROFILE-

Structure of Rivastigmine:



Chemical Name : Rivastigmine
Molecular Formula : $C_{14}H_{22}N_2O_2$
Molecular weight : 250.337 g/mole
Melting point : 158^0 C
Category : Cholinersterase inhibitorsDescription : use to treat mild to moderate dementia
Solubility : verily soluble in water and ethanol, and soluble in organic solvents

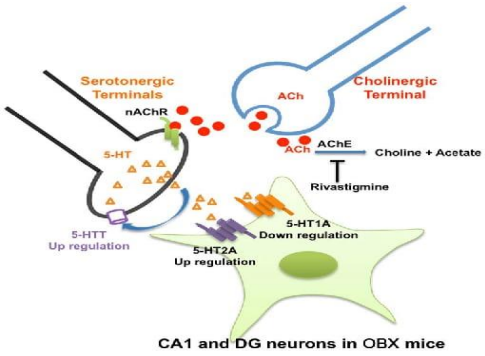
Mechanism of Action:

1. Inhibits Acetyl-cholinesterase (Ache) and Butyrylcholinesterase (BuChE)Rivastigmine prevents the breakdown of acetyl choline (ACh) by inhibiting these enzymes.

This increases acetyl-choline levels in the brain, improving cholinesterase neurotransmitter.

2. Enhances Cholinesterase Function

Acetyl choline is important for memory, learning, and cognition.By prolonging ACh activity, Rivastigmine helps slow cognitive decline in dementia.



Mechanism of action of Rivastigmine

Uses :

Rivastigmine is a medication used to treat:

1. Alzheimer's disease – Helps improve memory, awareness, and daily functioning in patients with mild to moderate Alzheimer's.

2. Parkinson's disease dementia – Used to manage cognitive symptoms in Parkinson's-related dementia.

MATERIALS AND INSTRUMENTS-

The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier with out further purification or investigation.

S.NO	MATERIALS	SOURCE
1	Rivastigmine	Swefn Pharmaceuticals private Ltd, Ahmedabad
2	Methanol	Merck Mumbai Ltd, Mumbai.
3	Ethanol	Merck Mumbai Ltd, Mumbai.
4	Sulphuric acid	Merck specialities Pvt Ltd, Mumbai.
5	Glacial acetic acid	Merck Mumbai Ltd, Mumbai.
6	Hydrochloric acid	Nice Chemicals Pvt Ltd, Kerala
7	Water	Nishanth, Kurnool.
8	Nitric acid	Merck specialist Pvt Ltd, Mumbai.
9	Chloroform	Merck specialist Pvt Ltd, Mumbai.

Instruments Used

- Citizen Electronic precision balance
- Digital Pro Ultrasonicator
- Analytical Technologies Limited 2080N Spectrophotometer.

METHODOLOGY-

UV SPECTROSCOPY

An Analytical Technologies Limited 2080N Spectrophotometer was used with 1 cm matched quartz cells. The data processing was performed using Analytical Technologies software.

UV METHOD DEVELOPMENT

The parameters for the development were as follows:

1) Selection of solvent

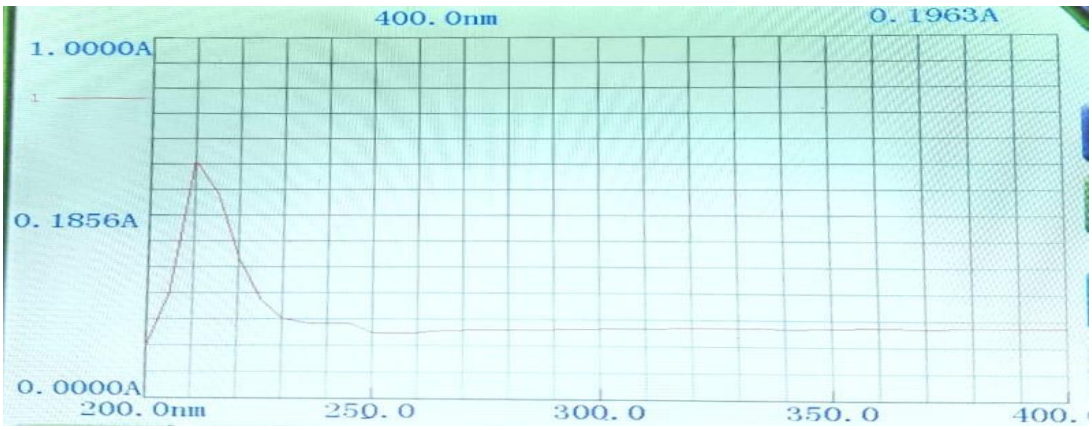
2) Selection of wavelength

Selection of solvent:

In order to select suitable solvent for determination of Rivastigmine, various solvents like 1N Hydrochloric acid, ethanol, 0.1N NaoH, DMSO[dimethyl sulfide], water, Acetate buffer, 2M hydrochloric acid, isopropyl, chloroform and methanol were tried for the solubility studies and it was found that Rivastigmine was soluble in 1M HCL, so in present investigation 1M HCL was selected as a solvent.

Selection of wavelength:

10µg/ ml of Rivastigmine solution was scanned in the range of 200-400 nm. The maximum absorbance was found at 209 nm.



Preparation of Solvent (1M HCL)

Take 36.5 ml of concentrated HCL and transfer it into the 1000 ml volumetric flask and make up the volume up to 1000 ml with the water.

Preparation of stock solution

A stock solution of Rivastigmine was prepared by accurately weighing 100 mg of drug, &transfer it in to 100 ml volumetric flask and dissolve it with 1M HCL and make up the volume to 100 ml with solvent 1M HCL (1000 µg/ml) name it as solution-1.

Preparation of working standard solution

An accurately measured quantity of 10 ml of standard stock solution-1 was transferred to 100 ml volumetric flask and diluted up to 100 ml with 1M HCL to give a working standard solution having strength of (100 µg/ml) and consider it as solution-2.

An accurately measured quantity of 10ml of solution-2 was transferred to 100 ml volumetric flask and diluted up to 100 ml with 1M HCL to give a working standard solution having strength of (10µg/ml) and consider it as working solution.

Validation of method-

Once the UV method development was over, the method was validated in terms of parameters like linearity, accuracy, precision, ruggedness and robustness.

Linearity

To evaluate the linearity, serial dilution of analyte was prepared from the working solution was diluted with solvent to get a series of concentration ranging from 1, 2,3,4,5,6 µg/ml.

The prepared solutions were filtered through Whitman filter paper (No.41). Calibration curve was constructed by plotting the absorbance on Y-axis against the concentration on X-axis. The results which are given in **Table 7.2** were within acceptable limits.

1µg/ml: 1 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to the 10ml with solvent 1M HCL.

2µg/ml: 2 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent 1M HCL.

3µg/ml: 3 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent 1M HCL.

4µg/ml: 4 ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent 1M HCL.

5µg/ml: 5ml of working standard solution was taken in 10 ml of volumetric flask.

6µg/ml: 6ml of working standard solution-3 was taken in 10 ml of volumetric flask diluted up to 10ml with solvent 1M HCL.

Acceptance criteria: correlation coefficient should not be less than 0.99.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of Rivastigmine (5µg/ml) were spiked with 80, 100, and 120 % Rivastigmine standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery of the pure drug. % RSD were calculated and reported .

Procedure

The standard solution was analyzed for three times and then each level of sample solution was analyzed in replicate for three times and the absorbance of standard and sample solutions was compared.

Precision-

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision is expressed as standard deviation or relative standard deviation.The precision of the method was demonstrated by Intra-day ~~and~~ inter-day variation studies.

Ruggedness-

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from analyst to analyst. The ruggedness of the method was determined by carrying out the experiment by different operators. The results of ruggedness testing are reported.

Robustness -

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and response was evaluated. Variation of wavelength (207 and 211 nm) had no significant effect on the absorbance of 4µg/ml solution, indicating that the method was robust.

RESULTS AND DISCUSSION:

Characteristic parameters of Rivastigmine for the proposed UV method

Parameters	UV
Calibration range (µg / ml)	1-6µg/ml
Wavelength(nm)	209
Regression equation (Y*)	0.0105x+0.2417
Intercept	0.2417
Slope (b)	0.010
Correlation coefficient (r^2)	0.9975
LOD (µg / ml)	1.6019
LOQ (µg / ml)	2.854

*Y = bx + a where x is the concentration of Rivastigmine in µg / ml and Y is the absorbance at the respective λ_{max}.

VALIDATION OF ANALYTICAL METHOD:

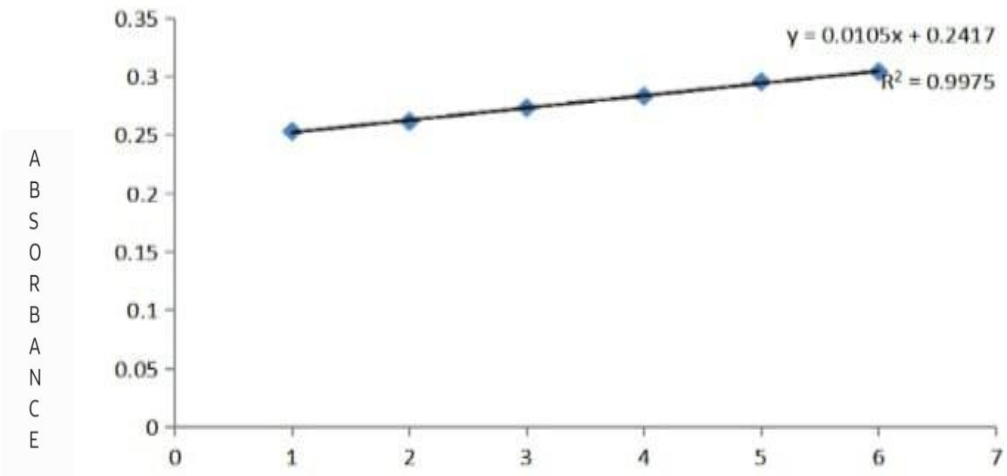
Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters.

1. Linearity

Calibration graph was plotted using absorbance of standard drug v/s concentration of standard drug solutions. Linear regression data showed a good linear relationship over a concentration range 1-6µg/ml.

Calibration curve for Rivastigmine

S. No.	Concentration(µg/ml)	Absorbance
1	1	0.2531
2	2	0.2615
3	3	0.2732
4	4	0.2832
5	5	0.2956
6	6	0.3041



Calibration curve of Rivastigmine

Observation:

1. The correlation coefficient for Rivastigmine was found to be 0.9975respectively.
2. The Linearity range for Rivastigmine was found to be 1-6µg/ml.

2. Accuracy

To study the accuracy of the method, recovery studies were carried out. The concentration of drug present in resulting solution was determined using developed procedure and percentage recovery and percentage RSD were calculated.

Accuracy summary

Sample (%)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered* (µg/ml)	%Recovery ± STDEV*	%RSD
80%	4	0.5	99.98	99.98±0.0027429	1.28
100%	5	0.5	100.3	100.3±0.0028919	1.71
120%	6	0.5	101.53	101.53±0.0017088	1.42

*Average of three determinations

Acceptance criteria:

1. % recovery should be within the range of 98-102%
2. % RSD should be not more than 2%

3. PRECISION

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation.

Intradayprecision results for Rivastigmine

S. No.	Concentration(µg/ml)	Absorbance
1	4	0.2674
2	4	0.2858
3	4	0.2909
4	4	0.2894
5	4	0.2851
6	4	0.2683
	STDEV	0.001053124
	AVG	0.28115
	%RSD	1.24

Inter day precision results for Rivastigmine

S. No.	Concentration(µg/ml)	Absorbance
1	4	0.2729
2	4	0.2908
3	4	0.2898
4	4	0.2865
5	4	0.2832
6	4	0.2365
	STDEV	0.02068404
	AVG	0.27661667

Acceptance criteria:
% RSD of the six replicate injections should not more than 2.0%.

4. Sensitivity:
Limit of detection (LOD) and limit of quantitation (LOQ) were determined from standard deviation and slope method as per ICH guideline, for Rivastigmine.LOD was found to be and LOQ was found to be

Observation of Limit of Detection

S. No.	Slope	STDEV of precision	LOD
1	0.010	0.0206	1.6019

Observation of limit of quatification

S.NO	SLOPE	STDEV	LOQ
1	0.010	0.0206	2.854

RUGGEDNESS:
Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

S. No	Analyst-1		Analyst-2	
	Con.(µg/ml)	Absorbance	Con.(µg/ml)	Absorbance
1	4	0.2729	4	0.2134
2	4	0.2908	4	0.2638
3	4	0.2798	4	0.2179
4	4	0.2265	4	0.2539
5	4	0.2332	4	0.2654
6	4	0.2539	4	0.2786
	STDEV	0.02601303	STDEV	0.02691681
	AVG	0.25951667	AVG	0.24883333
	%RSD	1.49	%RSD	1.54

Ruggedness studies of Rivastigmine by UV- visible spectroscopic method

Acceptance criteria:

1) % RSD should not more than 2.0%.

6. ROBUSTNESS:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Wavelength:

The solution was prepared and observed in replicate for six times with (±2) wavelength i.e. 207nm and 211nm respectively.

FOR wavelength
Observation for 207 nm and 211 nm wavelength

S. No	207 nm		211 nm	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	4	0.260	4	0.278
2	4	0.2623	4	0.2803
3	4	0.2703	4	0.2826
4	4	0.2611	4	0.2708
5	4	0.2634	4	0.2638
6	4	0.2701	4	0.294
	STDEV	0.004457	STDEV	0.0081778
	AVG	0.2646	AVG	0.27338333
	%RSD	1.34	%RSD	1.69

Robustness summary for wavelength

S. No	Condition	Modification	Mean Absorbance ± STDEV	% RSD (for Absorbance
1	Wavelength (nm)	207	0.2646 ±0.004457	1.34
		211	0.2733±0.0081778	1.69

DISCUSSION:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low-cost UV-Visible Spectroscopic method. It is successfully applied for the determination of Rivastigmine in pharmaceutical preparations without the inferences of other constituent in the formulations.

The optimum wavelength for detection was 209nm at which better detector response for the drug were obtained. The calibration was linear in concentration range of 1-6µg/ml for Rivastigmine in the Table No: 7.2 respectively**.** The sensitivity for the drug has been calculated and the LOD and LOQ of the Rivastigmine was found to be1.6095µg/ml and 2.854 µg/ml in the Table No: 7.10 & 7.11 respectively.

The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 98.3-101.1% for Rivastigmine in the Table No:7.7 respectively.
Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % R.S.D. reported was found to be less than 2 % respectively.

CONCLUSION:

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision.

There are few analytical methods appeared in the literature for the determination of the Rivastigmine. In view of the above, a simple and specific analytical method was planned to develop with sensitivity, accuracy, precision and economical.

In the present investigation of, UV method for the quantitative estimation of Rivastigmine in pharmaceutical dosage form has been developed and validated.

The proposed UV method is more sensitive, accurate and precise and is suggested for routine analysis.

REFERENCES:

1. S. Alexandar, Rohini Diwedi, T. Ashok and M. J. N. Chandrasekhar- A validated RP-HPLC

method for estimation of Rivastigmine in pharmaceutical formulations published in "Scholars Research Library" 2011; 3(3):421-426.

2. Hossein Amini and Abolhassan Ahmadiani-High-Performance Liquid Chromatographic Determination of Rivastigmine in Human Plasma for Application in Pharmacokinetic Studies published in " Iranian Journal of Pharmaceutical Research" 2010; 9 (2): 115-121.

3.Raja Sundararajan, Aarthi Kommu-Analytical Method Development and Validation of Rivastigmine in its Pure and Pharmaceutical Dosage form Using UPLC published in "International Journal of Pharmaceutical Investigation" 2023; 13(3):595-604.

4.Thota Devendra Sairam, G.Kishore Babu, P.Srinivasa Babu- Characterisation and Evaluation of Rivastigmine Loaded Transdermal Films for the Patients Enduring with Alzheimer's disease published in "Indian Journal of Research in Pharmacy and Biotechnology" 2015**;** 3(6):443-451.

5.M.N.Kale-Development of validated hplc method for quantitative estimation of Rivastigmine Hydrogen Tartrate in transdermal drug delivery system published in "International Journal of Pharmaceutical Sciences and Research" 2014; Vol. 5(5):1892-1902.

6.Ashwini R Walave , Hemlata S. Bhawar , Mayur S. Bhosale- Analytical Methods Development And Validation For Estimation Of Rivastigmine Drug Used For Alzheimer's Disease published in "International Research Journal of Engineering and Technology" 2023; 10(1): 04: 465-471.

7. Deepshi Arora, Manish Kumar , Shailendra Bhatt , Yugam Taneja , Abhishek Tiwari and Varsha Tiwari- UV Spectrophotometric Method for Quantification of Rivastigmine Tartrate in Simulated Nasal Fluid development and validation published in "Biomedical & Pharmacology Journal" 2021 ; 14(4):2165-2172.

8.Karthik,Arumugam,Prashant,B Musambade ,Subramanian Ganeshan - Stability-Indicating HPTLC Determination of Rivastigmine in the Bulk Drug and in Pharmaceutical Dosage Forms published in " JPC - Journal of Planar Chromatography" 2007; 9 (2) 457-461.

9.Avijit Choudhury , K. Vasantakumar Pai , Suddhasattya Dey , Rajesh J. Mandade-RP-HPLC Method For The Estimation of Rivastigmine in Bulk and in Dosage Forms published in "Journal of Pharmacy Research" 2011,4(4);1007-1009.

10.Aarthi Kommu, Raja Sundararajan-Analytical Method Development and Validation of Rivastigmine in its Pure and Pharmaceutical Dosage form Using UPLC published in "International Journal of Pharmaceutical Investigation" 2023; 13(3):595-604.