A RAPID, SIMPLE AND SENSITIVE METHOD OF RIVASTIGMINE TARTRATE BY VISIBLE SPECTROPHOTOMETRIC METHOD

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Abstract

In the present investigation, the **Rivastigmine Tartrate** drug was used for method development and validation in quality control analysis. A simple, sensitive, and accurate with good precision method was developed by using the visible spectroscopic technique for stability and estimation of the drug in its pure and formulation forms. The process used for the method was simple and inexpensive. For method validation, the linearity, detection limit and quantification, repeatability, intraday and inter-day precision, ruggedness and accuracy characteristics were studied. The equation of calibration curve obtained was y = 0.0073x-0.0051. The correlation coefficient (r) was found to be 0.9998.The developed method was expressed good reproducibility and recovery with % RSD less than 2.The obtained results were shown that from the proposed method was found to be simple, sensitive, accurate and with good precision. Thus, this approach could be considered for the analysis of this drug in quality control laboratories.

Key words: Rivastigmine tartrate, visible spectroscopy, linearity, and precision**.**
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Introduction

Rivastigmine Tartrate contains not less than (NLT) 98.0% and not more than(NMT) 102.0% of the labeled amount of $C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$, calculated on the anhydrous basis. The chemical name was Ethyl methyl carbamic acid, 3-[(S)-1-(di methyl amino)ethyl]phenyl ester, (2R,3R)-2,3-dihydroxybutanedioate; (S)-3-[1-(Di methyl amino)ethyl] phenyl ethyl methyl Carbamate[1], hydrogen tartrate. The molecular weight of Rivastigmine tartrate is 400.42 which contain inorganic and organic impurities and also can be identified by Infrared Absorption for structural conformation. The drug could be preserved in tight containers, and store at room temperature. The structure of Rivastigmine Tartrate drug is as follows.
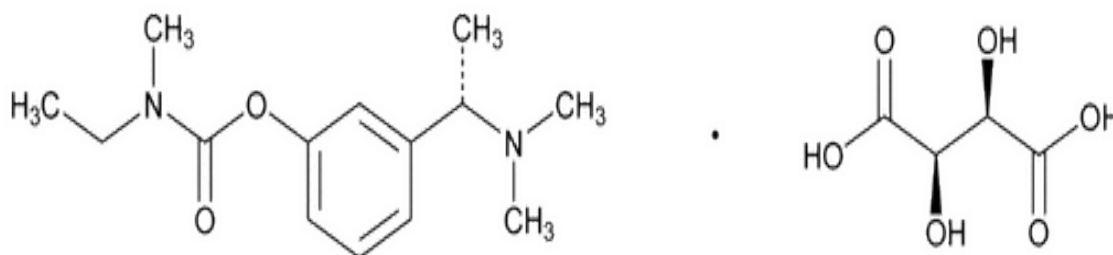

Fig.1. Chemical Structure of Rivastigmine Tartrate

Rivastigmine is used to treat confusion (dementia) related to Alzheimer's disease and to Parkinson's disease. Rivastigmine does not cure either of these diseases, but it may improve memory, awareness, and the ability to perform daily functions[2]. This medication works by restoring the balance of natural substances

(neurotransmitters) in the brain. Take this medication by mouth with fed (after taking food) condition as directed by doctor, usually twice in the day. It shows high brain region selectivity for the hippocampus and spectrophotometric method[3], is used techniques for the determination of Rivastigmine.

In general, most of the regulatory authorities recommend the presence of impurities to be 0.1%, and any level above should be identified and quantified through appropriate analytical techniques[4]. A through literature review indicated that the availability of few analytical methods for determination of the drugs by spectroscopic methods [5, 6] i.e., U.V. visible method. However, quantification of Rivastigmine Tartrate in pure form and its dosage form is a newer approach and has not been so far. Ultraviolet-visible spectrophotometer investigates the interaction of light radiation with matter in the ultra violet (200-400nm) and visible (400-800 nm) range[7].

Depending on the API, not every stress agent may affect degradation, but each agent has to be evaluated to determine whether degradation occurs[8].Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. Forced degradation studies provide data to support identification[9,10]. The UV-Visible spectroscopic data can give qualitative and quantitative information of a given compound or molecule[11,12].

Principle

This method is based on the principle that folinciocalteu(FC)reagent is useful for the detection of amines and forms a colored species in the reaction. FC reagent gets reduced and the oxidation of active ingredient produces bluish-green chromogen. The reaction takes place in the presence of a basic medium.

FC reagent is a mixture of poly hetero acids i.e., poly tungstic acid and poly molybdic acid. The hetero acids are mixed in 1:1 ratio to produce folinciocalteu reagent. From the below reaction scheme, results the oxidation of the drug compound after which the reagent is reduced to a mixture of blue-green oxides of tungsten, and Molybdenum.

Reaction
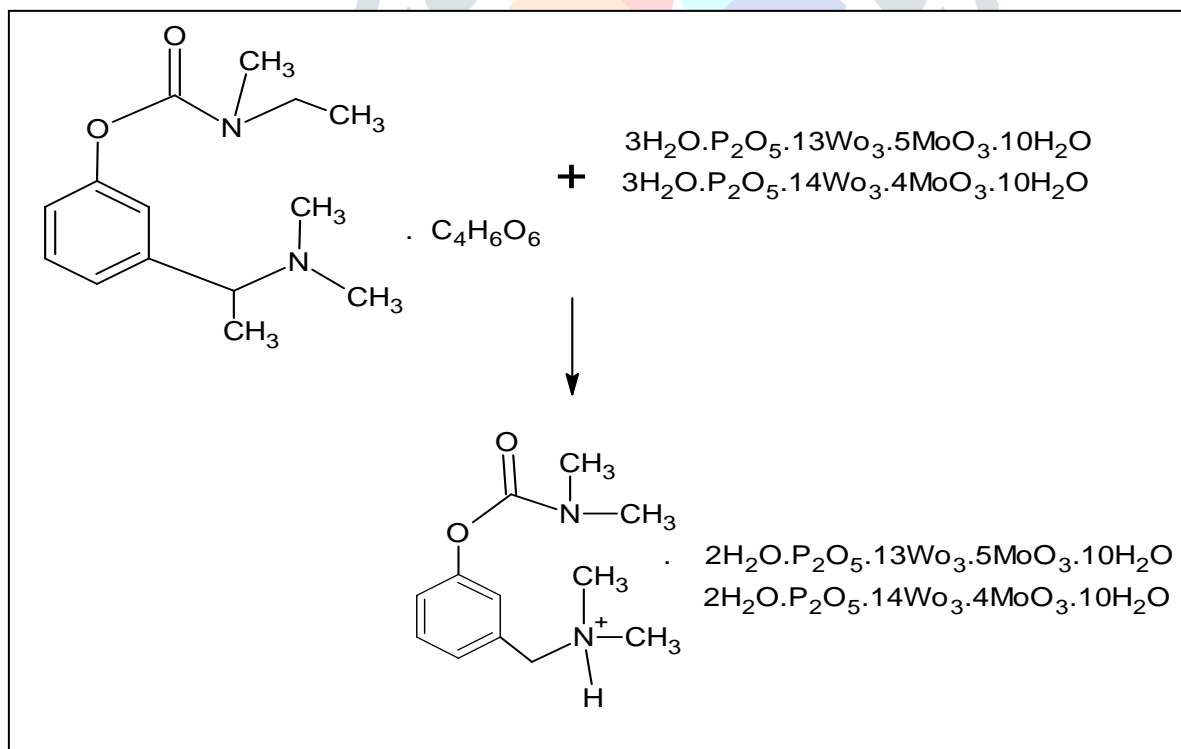
Bluish green chromogen at λ$_{max}$725 nm

Mechanism

The mechanism involves the formation of ion that is the bond between the reagent and the drug. The drug moiety consists of two nitrogen atoms among which each nitrogen atom bonded to three methyl groups

participates in ion pair formation. It loses electrons and gets oxidized and later the reagent gets reduced resulting in a bluish green chromogen. The rate of reaction depends upon various factors. They are,

1. The concentration of the basic medium (sodium carbonate solution)
2. The concentration of FC reagent
3. pH of the medium
4. Order of the addition

Materials and Methods

The Spectrophotometric measurements were carried-out using a Shimadzu UV-1700 UV/Vis spectrophotometer with 1cm matched quartz cell and Shimadzu ELB 300 analytical balance, Rivastigmine tartrate pure drug (99.95%) was obtained as a gift sample from Sun Pharmaceutical Industries Ltd. All chemicals and reagents used were of analytical grade. The formulationdrug used for this study was developed by Sun Pharmaceutical Industries Ltd, India.

Preparation of Standard solution

Standard drug of Rivastigmine tartrate was proposed by dissolving 100mg pure Rivastigmine tartrate in distilled water and transferred into the 100ml volumetric flask to obtain 1000μg/ml of stock solution. About 1ml of the above solution was pipette out into a 10ml volumetric flask and 1ml of FC reagent was added to it. To this solution, 3.5ml of 10% sodium carbonate solution was added. The solution was allowed to stand for 10minutes. Finally, the volume made up to 10ml with distilled water. The final concentration of this solution was 100μg/ml. The standard solution of Rivastigmine tartrate having a concentration of 100μg/ml, and was scanned in UV-VIS range (400-800nm) in 1.0 cm cell against in solvent as blank and the spectrum was obtained.

Determination of λmax

100μg/ml of Rivastigmine tartrate was prepared and scanned in UV-VIS range of 400-800nm, and spectrum was recorded. The λmax was found to be at a 725nm wavelength where absorbance was found maximum at this wavelength. Hence it is considered as absorbance maxima (λmax).

Preparation of calibration curve

The standard stock solution was suitably diluted with ethanol to obtain concentrations ranging from 25-150μg/ml. The absorbance of these solutions was measured at 725nm. A calibration curve was obtained by plotting graph between concentrations versus absorbance.

Preparation of test solution

The 20 Tablets were weighed and its average weight was determined. An accurately weighed tablet powder equivalent to 100mg of Rivastigmine tartrate transferred into 100ml volumetric flask dissolved in distilled water, sonicated for 10min and volume was made up to the mark. The solution was filtered using Whatman filter paper (No.41) and from that 1ml filtered solution was transferred into a 10ml volumetric flask and made up to the mark with distilled water to obtain 100μg/ml solution.

Method Development

Selection of reagent

For the visible spectrophotometric method, various reagents were investigated. The drug reacts with folin-ciocalteu reagent as it has an amine group. The reaction results in the formation of a bluish green chromogen under basic conditions which shows λ_{max} at 725 nm. Finally, the method has been optimized by using 10% sodium carbonate solution and FC regent using double distilled water as diluent.

Preparation of 10% Na_2CO_3

About 10 grams of Na_2CO_3 (analytical grade) was weighed into a 100ml beaker. The compound was dissolved using double distilled water and was later diluted to 1000ml with double distilled water in a volumetric flask.

Standard solution preparation

About 100mg of Rivastigmine tartrate was weighed and transferred to a standard flask. About 20ml of the distilled water was added and the solution was sonicated for 10minutesto dissolve the solution. The volume was made up to 100ml with the same diluent (1000μg/ml). About 1ml of the above solution was pipette out into a 10ml volumetric flask and 1ml of FC reagent was added to it. To this solution, 3.5ml of 10% sodium carbonate solution. The solution was allowed to stand for 10minutes at room temperature. Finally, the volume made up to 10ml with distilled water. The final concentration of the solution was 100μg/ml. The baseline was adjusted to zero using blank which is a solution of 1ml FC reagent and 3.5 ml

of sodium carbonate made up to 10ml with water. The spectrum was recorded and the λ_{max} of the final solution was found at 725nm.The spectrum is shown in **Fig.2**.

Procedure for pharmaceutical preparation

Twenty capsules of the marketed formulation of Rivastigmine tartarate were accurately weighed and the fine powder within the capsules was taken out carefully. An equivalent quantity of powder was transferred into a 25ml capacity of the volumetric flask containing 25ml of double distilled water which was sonicated for 15 min and made up to the mark with diluent. The above solution was filtered through Whatman filter paper and later through an Injectable filter (0.45μ). The filtrate was diluted to get a final concentration of 100μg/ml. Further dilutions were made from the above-prepared solution. All determinations were conducted in triplicate and the absorption spectrum was recorded at 725 nm.

Method Validation

Linearity

The absorbance's were observed from 25to 150μg/ml and were shown in Table1. Linearity was obtained between 25to 150μg/ml. The concentration graph was plotted for concentration and absorbance. The equation of calibration curve obtained was y = 0.0073x - 0.0051. The correlation coefficient (r) was found to be 0.9998.

Precision

Repeatability

The six concentrations of 100μg/ml were prepared and the absorbances were read. The % of RSD was calculated.

Intraday and Inter-day Precision

The concentration of 50μg/ml, 100μg/ml and 150μg/ml of Rivastigmine tartrate (on label claim basis) were taken, the absorbance of the final solution was read after 0hr, 12hr and 24hr in 1.0 cm cell at a selected wavelength. Similarly, the absorbance of the same solutions was read on the 1st and2nd day. All the solutions were prepared as triplicate and analyzed them.

Accuracy

To determine the accuracy of the method recovery was performed by standard addition method. To pre-analyzed sample known the amount of standard Rivastigmine tartrate was spiked in different concentrations. The recovery was performed at three levels 50%, 100% and 150% of standard Rivastigmine tartrate. The solutions were prepared in triplicate and the accuracy was indicated by % Recovery.

Ruggedness

It was carried out by analyzing the sample by two analysts and estimation of the drug by proposed methods. The % of RSD was calculated.

Results and Discussion

An attempt has been made to develop rapid, sensitive, economic, precise and accurate analytical method for Rivastigmine tartrate in the pure and pharmaceutical dosage form. The proposed method is based on UV Spectrophotometric absorption in the visible region (400-800nm). A graph is drawn between wavelength and absorbance which is shown in Figure 1. The maximum absorbance was found to be at 725nm and is shown in **Figure 2**. Beer's law was obeyed in concentrations ranging from 25to 150μg/ml is incorporated in **Table 1**. The calibration curve is obtained by graph drawn between concentration and absorbance and is shown in **Figure 3.** The correlation coefficient values were above 0.9998which shows that absorbance was linear with concentration(**Figure 3**). It is seen that Rivastigmine tartrate drug is showed the linearity.
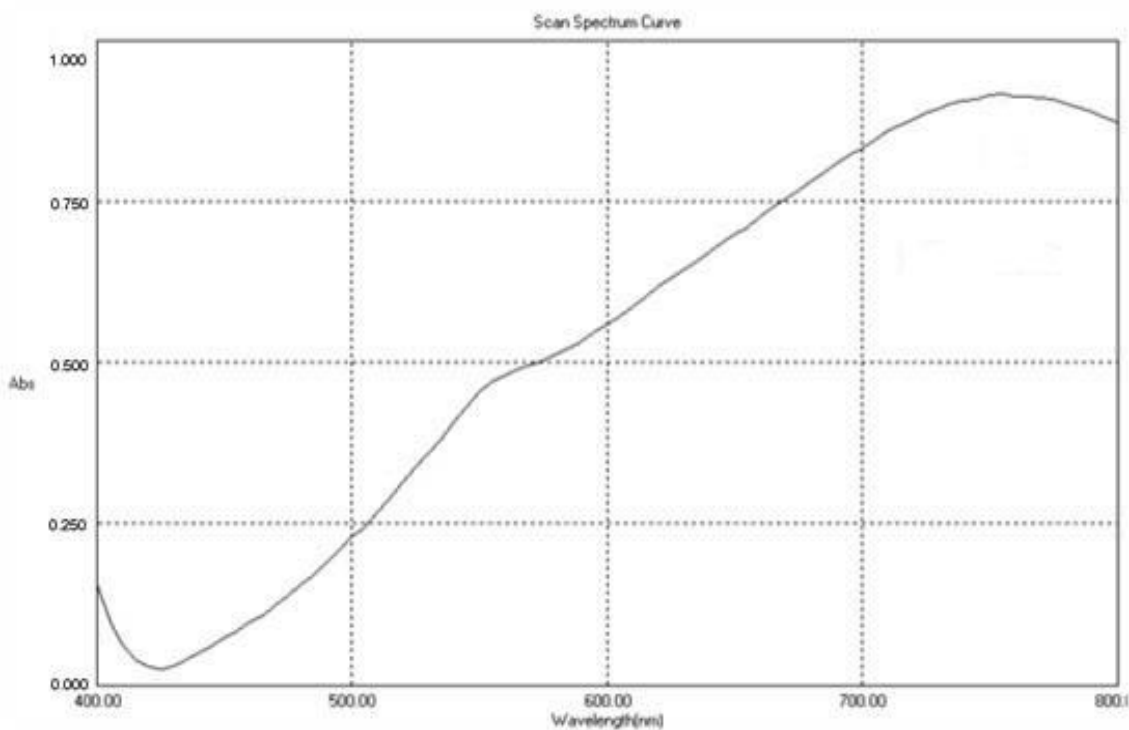
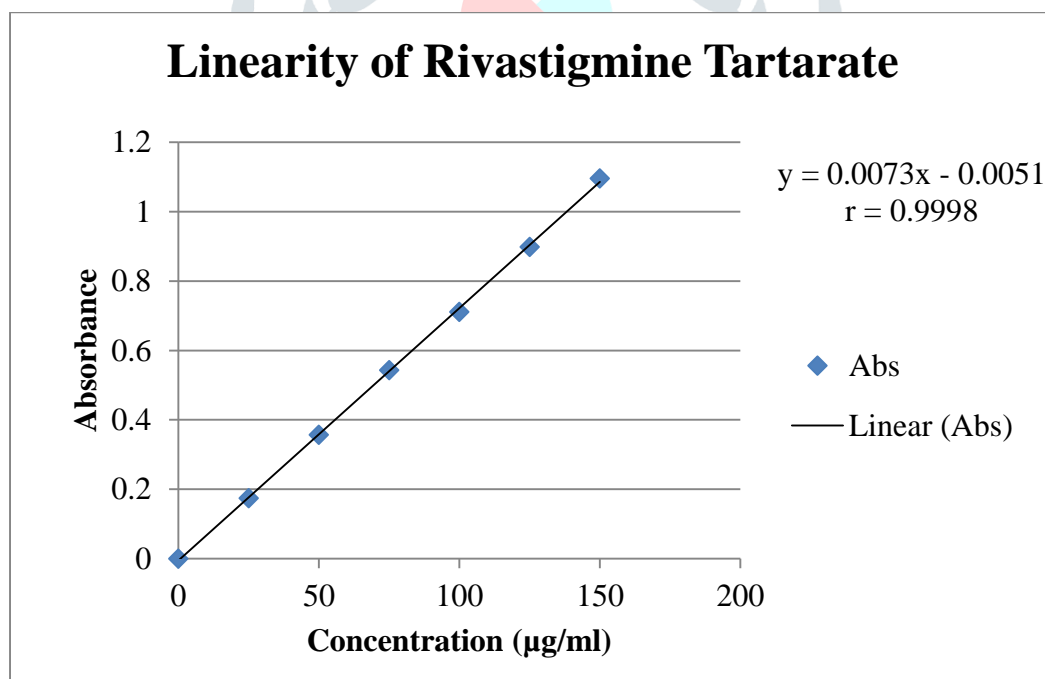**Fig. 2. UV-Visible Spectrum of Rivastigmine tartrate at λmax = 725nm**

Fig. 3. Calibration curve of Rivastigmine tartrate showing linearity relationship

Table 1. Linearity data for analysis of Rivastigmine tartrate

S.No.	Concentration (µg/ml)	Mean Absorbance ($\pm$SD)*
1	25	0.175 ($\pm$ 0.001)
2	50	0.356 ($\pm$ 0.001)
3	75	0.543 ($\pm$ 0.0012)
4	100	0.712 ($\pm$ 0.001)

| 5 | 125 | 0.898 ($\pm$ 0.0006) |
| 6 | 150 | 1.097 ($\pm$ 0.001) |

*n=3 (Average of 3 determinations)

The precision of the method was confirmed and precision by intraday and inter-day analysis also confirmed and the % of RSD values were represented in **Table 2, 3 and 4.** The precision of the UV analytical method is determined by carrying out the analysis as per the procedure and as per normal weight is taken for analysis. Repeat the analysis for six times. Calculate the % assay, mean assay, and % relative standard deviation.The developed method was found to be precise as the %RSD values for the intraday and inter-day analysis precision studies were 99.25% and 99. 67%, respectively **(Table 3& 4).**

Table 2. Precision data for analysis of Rivastigmine tartrate

S.No.	Concentration (µg/ml)	Absorbance	%Assay	Mean Assay $\pm$ %RSD
1	100	0.706	99.11	
2	100	0.711	99.81	
3	100	0.709	99.53	99.39 $\pm$ 0.33
4	100	0.710	99.67	
5	100	0.705	98.97	
6	100	0.707	99.25	

Table 3. Results of Intraday Precision of Rivastigmine tartrate

Parameter	% Recovery Estimated (Mean $\pm$RSD)*		
	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)
At 0 hr	99.76$\pm$ 0.71	99.81$\pm$ 0.42	99.95$\pm$1.07
At 12 hr	99.95$\pm$1.02	99.20$\pm$ 0.36	99.82$\pm$ 0.15
At 24 hr	100.32 $\pm$ 0.16	99.25$\pm$ 0.42	99.75$\pm$ 0.10

*n=3 (Average of 3 determinations)

Table 4. Results of Inter-day Precision of Rivastigmine tartrate

Parameter	% Recovery Estimated (Mean $\pm$RSD)*		
	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)
Day-1	99.48$\pm$ 0.71	99.67$\pm$ 0.51	100.04$\pm$1.05
Day-2	99.48$\pm$ 0.99	99.81$\pm$ 0.28	99.59$\pm$ 0.42

*n=3 (Average of 3 determinations)

The % of Recovery studies were performed at 50%, 100%, and 150% and were represented in **Table 5.** A standard quantity equivalent to 50%, 100%,and 125% is to be added in the sample. The resultswereshown that best recoveries (99.70-100.57%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate (Table 5). The limit of detection and limit of quantification were determined and were shown in **Table 6.** The LOD and LOQ were found to be 0.452µg/ml and 1.370µg/ml **(Table 6)**.The results were found to be satisfactory and are reported in Tables 5 and 6.

Table 5. Recovery data of Rivastigmine tartrate

Ingredient	Amount of drug from the	Amount of standard	Percentage added	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery (Mean $\pm$RSD)*

	formulation	added				
Rivastigmine tartrate	50µg	25µg	50%	25	25.14	100.57±1.50
Rivastigmine tartrate	50µg	50µg	100%	50	49.85	99.70± 0.90
Rivastigmine tartrate	50µg	100µg	150%	100	75.25	100.33± 0.70

*n=3 (Average of 3 determinations)

Table 6. Lowest Limit of detection and Lowest Limit of quantification

LOD (µg/ml)	LOQ (µg/ml)
0.452	1.370

The ruggedness parameter was performed between two analysts and % of RSD was found at 0.51 and 0.63. The results were indicated by % RSD and are shown in **Table 7**. The assay of the one brand was performed and the results were incorporated in **Table 8.**The results of the Assay show that the amount of drugwas in good agreement with the label claim of the formulation as indicated by % recovery (100.57%). The optical characteristics such as Beer's law limit, correlation coefficient, slope, intercept, molar absorptivity, scandell's sensitivity was calculated and validated **(Table 9)**. The method was validated and found to be simple, sensitive, accurate and precise. Hence the proposed method could be effectively adopted for routine quality control of Rivastigmine tartrate in bulk and formulated tablet dosage form.

Table 7. Results of Ruggedness of Rivastigmine tartrate

Ruggedness	%Purity ± RSD*
Analyst – 1	99.95±0.51
Analyst – 2	99.76± 0.67

*n=3 (Average of 3 determinations)

Table 8. Results of analysis of laboratory samples (Assay)

Sample	Label	Amount found	% Purity ± RSD*
RIVAMER	3mg	2.99mg	99.58± 0.43

*n=3 (Average of 3 determinations)

Table 9. Validation Parameters

Parameters	Results
Absorption maxima λmax (nm)	725
Beer's law limit (µg/ml)	25-150
Molar Absorptivity (L mole^{-1}, cms^{-1})	3.129 X 10^3
Sandell's sensitivity (µg/cm2/0.001)	0.1404
Correlation coefficient	0.9998
Regression equation	y = 0.0073x - 0.0051
Limit of detection	0.452
Limit of quantification	1.370
Precision(% RSD)	0.33

CONCLUSIONS

The Spectrophotometric method being reported for the assay of Rivastigmine tartrate in pure form and also in its formulations is simple and inexpensive. The method was statistically validated according to the ICH guidelines. Spectrophotometric method linear over response was obtained in the concentration range of 25–150 μg/mL, with a correlation coefficient of 0.9998.The maximum absorbance was found to be at 725nm.The proposed method was found to be simple, sensitive, accurate and with good precision. Thus, this approach could be considered for the analysis of this drug in quality control laboratories. Moreover, the described method was compatible with quality control department i.e., Ultraviolet visible analysis with less time and cost-effective in terms of this required fewer chemicals consumption with added advantages.

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