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UV Spectrophotometric Method for Quantification of Rivastigmine Tartrate in Simulated Nasal Fluid: Development and Validation

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Rivastigmine Tartrate belongs to the class of cholinesterase inhibitors in Anti-alzheimer's disease with optimum therapeutic efficacy. Till now no validated method of its quantification has been reported in simulated nasal fluid. The current research investigation aims to develop a rapid, simple, and reliable UV spectrophotometric method for the quantitative determination of the pure form of Rivastigmine Tartrate in SNF. A suitable method was developed by using double beam UV spectrophotometer and selection of a suitable solvent system for estimation of Rivastigmine Tartrate at absorbance maxima 263nm in SNF. The method was validated for various parameters like including accuracy, linearity and precision as per the International Conference on Harmonization guidelines. The method developed by selecting simulated nasal fluid as the solvent system satisfied the optimum condition of the good quality peak at the selected wavelength. The results proposed the developed method for Rivastigmine Tartrate quantification in the simulated nasal fluid to be linear in the working concentration range of 5-60 μg/ml with a co-relation coefficient of 0.998. The % accuracy was found to be 99.8 -100.57. The % RSD values were < 2 while LOD & LOQ values were detected to be 0.316 and 1.053 respectively. The stated method was analyzed to be rapid, accurate, reliable, and precise. Further, it can be used in checking the quality control parameters of the Rivastigmine Tartrate in routine analysis.

Keywords: Inter-Day; Intra-Day; Rivastigmine; Spectrophotometer; Simulated Nasal Fluid.

Rivastigmine Tartrate (RT), structure shown in Fig 1, is an acetylcholinesterase inhibitor that inhibits both the acetylcholinesterase and butyrylcholinesterase enzymes reversibly, which has been found to be superior in terms of specificity of action and minimal side effects[1].

Literature survey revealed that it is widely used for mild to severe stages of Alzheimer[2-3],

including treatment of dementia that is associated with Parkinson's disease as it improves cognition, ADL and global function[4]. RT was the first approved drug to be marketed for Alzheimer's in Switzerland in the year 1997, and then became popular in 80 countries worldwide, including the Canada, Europe etc[5]. RT cause minimal interactions when taken with other drugs especially in elderly individuals, who are on different medications for concurrent illness that increase its importance further in the market[6]. Though it is BCS class 1 drug and has both good solubility and permeability, still its use is limited due to its low bioavailability[7], poor penetration through BBB[8], short half-life and gastrointestinal side effects when administered orally[9]. It is freely soluble in a wide range of solvents[10] like distilled water, ethanol, acetone, phosphate buffers etc and shows absorption maxima at 263 nm. To solve problems like short half life, poor penetration across BBB[11] due to presence of efflux transporters and gastrointestinal disorders various lipid particulate systems like SLNs[12] and NLC[13], nanocarriers like polymeric nanoparticles, nanosponges have been developed.

As UV spectrophotometry is rapid, accurate and reliable[14] it continues to be a popular method for quantitative determination of drugs[15]. The research aimed to formulate an intranasal delivery of RT to bypass the BBB for its increased bioavailability so the solubility of drug in SNF was eminent and it is evident from the literature review that there is lack of simple method for UV determination of RT in simulated nasal fluid[16-17]. Therefore, the current research findings aim to develop and validate a method using UV-Visible spectrophotometer in SNF for the estimation of RT.

MATERIALS AND METHODS

Chemicals

RT was procured as a gratis sample from Alembic Pharmaceuticals (Vadodra, Gujarat). Potassium chloride, Sodium chloride and Calcium chloride were purchased from Sigma Aldrich, Bangalore, India. The other reagents that were used were of analytical grade. Double distilled water was used.

Method development

Instrumentation

The spectrophotometric analytical determination of RT was done by using UV- Visible Spectrophotometer (Kyoto, Japan)[18] having model no.1601 with a pair of quartz cells and 10 mm path length.

Preparation of SNF

Simulated nasal fluid was prepared by dissolving accurately weighed (1.29 mg/ml) KCl, (7.45 mg/ml) NaCl, (0.32 mg/ml) $CaCl_2.2H_2O$ in 1000 ml of distilled water. Orthophosphoric acid was used to adjust the pH of solution to 6.35.

Standard stock solution

Stock solution I

Primary standard stock solution of 1mg/ml(1000 µg/ml) of RT was prepared by dissolving pure drug 25 mg in a volumetric flask of 25 ml with SNF and the resultant solution will give the concentration of 1000 µg/ml . Primary stock solution was stored at refrigerating conditions.

Stock solution II

From the primary standard stock solution prepared, aliquot of 1ml was transferred to 10 ml of volumetric flask to get a secondary stock solution with concentration of 100µg/ml. After that different test solutions of concentration (5–60)µg/ml was prepared by transferring aliquots to a 10 ml volumetric flask and their volume was made up with SNF.

Determination of lambda max (λ_{max})

RT-test solution of 50 µg/ml was prepared and then scanned at a wavelength of 200 to 400 nm against the blank. The λ_{max} obtained for the prepared solution was noted where absorbance was found to be maximum and considered as absorption maxima which will be used used for preparation of the calibration curve.

Calibration Curve of Rivastigmine Tartrate in SNF

RT calibration curve was prepared in SNF. The test solutions prepared were shaken properly before checking the absorbance at 263nm (Fig 2) against SNF as the blank solvent. The procedure was performed in triplicate and the mean absorbance was noted.

Analytical method Validation:

Linearity [19]

Linearity is the ability of an analytical method to produce the observed concentrations results of the tested samples proportionally to the theoretical concentration of analyte in the measured samples either directly or by a suitable

mathematical transformation. The calibration curve of abs. vs conc. was plotted in which dependent variable (absorbance Y) was taken on Y axis as a function of an independent variable (concentration X) on X-axis.

Accuracy[19]

An analytical method is said to be accurate if the obtained test results are closer to the theoretical value. % Analyte recovery studies is performed at three levels 50,100 and 150 % and the test solutions are analyzed by the proposed methods and observed concentrations are back calculated using the regression equation obtained from the prepared calibration curve and compared with the theoretical concentrations.

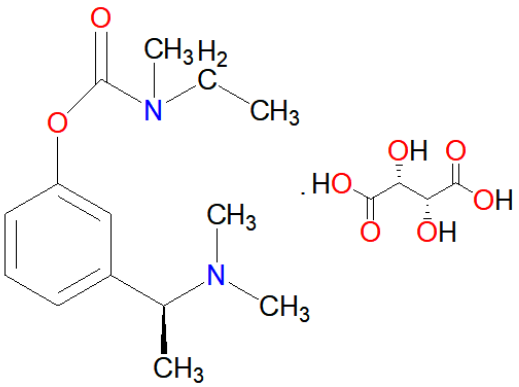

Fig. 1. Structure of Rivastigmine Tartrate

Precision

Precision of the developed method is done to check the reliability, reproducibility and repeatability in case the same analytical procedure is applied repeatedly used on the same sample under normal experimental conditions agreement among individual test results when the procedure is applied repeatedly to multiple sampling of homogenous sample[10]. Inter- day and intra-day precision of the samples was carried out at three quality control concentrations (Low, Medium, High) and the variations in the results are expressed as %RSD[20].

Limit of Detection (LOD)

LOD can be quantified as the minimal analyte concentration that is detectable in a sample under given experimental conditions with acceptable degree of precision and accuracy. The values of LOD for a given sample are specific under specified conditions and for a given set of experimental conditions. Its a quantitative method whose values changes with the change in method, instrument etc[20].

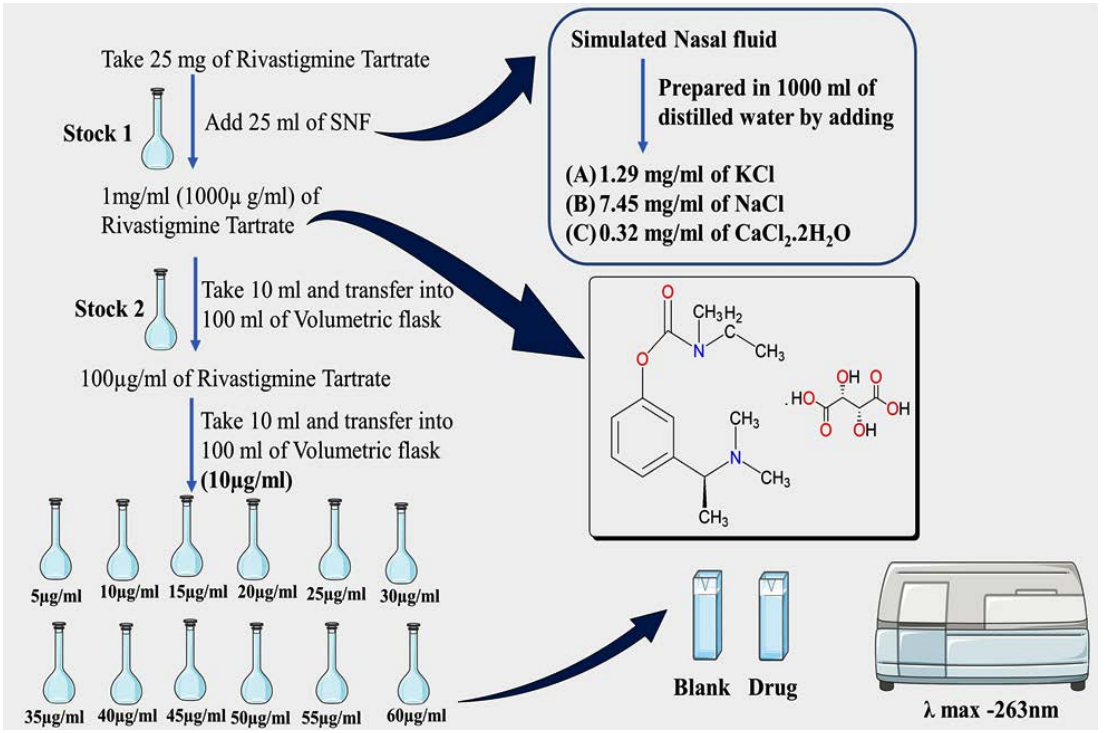

Fig. 2. Preparation of RT test solutions (5-60 µg/ml) in SNF

LOQ is the lowest analytical concentration of a tested sample within a sample set that may be measured and its value is almost 10 times higher than that of the blank.

Robustness

The robustness of the method was determined by selecting concentration of RT-test solution of 30 µg/ml as working concentration and changing the wavelength by 263 nm.

Repeatability

Repeatability of RT-test solution was also determined by the same working concentration (30 µg/ml) for six times.

RESULTS AND DISCUSSION

Determination of UV absorbance maxima and preparation of calibration curve

The maxima absorption of the RT-test solution was obtained at 263 nm which has not

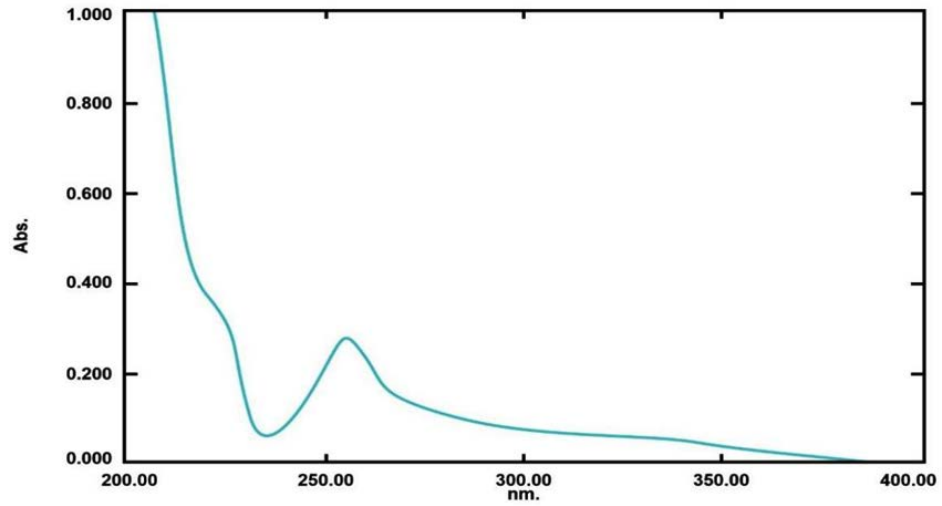

Fig.3. Absorbance maxima of RT in SNF at 263 nm

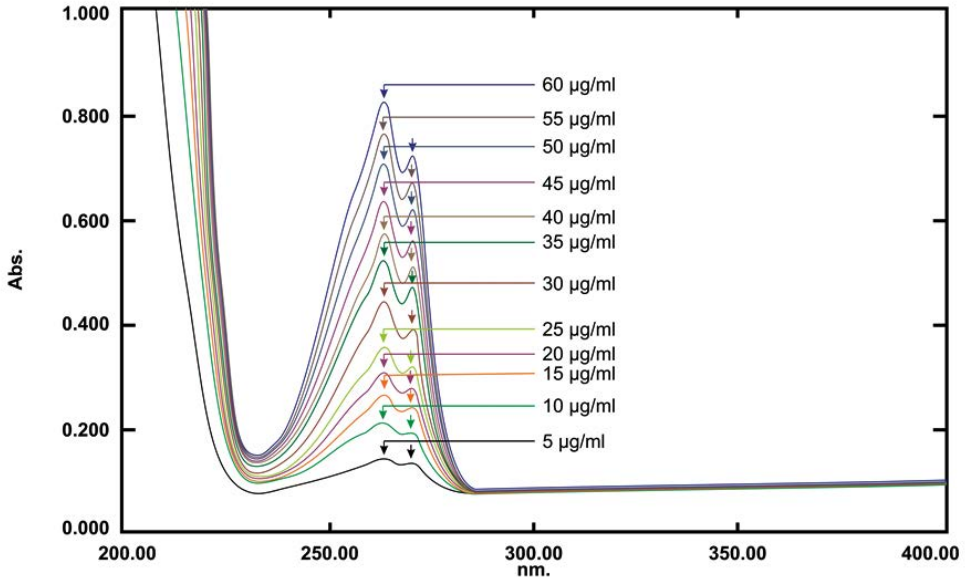

Fig. 4. Overlay plot of RT test solutions (5-60) µg/ml in SNF

been reported earlier in SNF and can be seen in the Fig 3. The test solutions were scanned at different concentrations of the RT-solution and overlay plot was obtained as shown in the Fig 4.

Linearity

The data obtained for linear regression of RT-test solutions over the range of (5– 60) µg/ml shows good linearity in the range of (5-60) µg/ml with low limits of standard deviation that reveals that solutions have good consistency. The linear regression equation was found to be y = 0.017x -0.023 and R^2 = 0.998 as shown in Fig 5 and Table 1.

Accuracy

The accuracy of the re-analyzed RT-Test solutions prepared in SNF ranged from 99.8-100.57% as recorded in the table.The results of recovery studies suggest the accuracy of method as shown in Table 2.

Precision

The %RSD results for intra- and inter day variability are typically low i.e <2% and <1% respectively of the absolute value which signifies that the developed method for estimation at all the concentration level is optimum. The results for both the inter-day and intra-day precision are shown in Table 3 and Table 4 respectively.

Table 1. Linearity results of RT test solutions in SNF

Simulated nasal Fluid	5-60 µg/ml	3	5-60 µg/ml	y = 0.017x -0.023	R^2 = 0.998

Table 2. % Accuracy results of RT test solutions in SNF

Theoretical Concentration (µg/ml)	Calculated Concentration (µg/ml)* (±SD)	% Accuracy
5	5.02 ± 0.0013	100.40
30	29.96 ±0.0015	99.8
60	60.32 ±0.0025	100.57
Average		100.2

LOD and LOQ

The sensitivity of the proposed method was detected by LOD and LOQ whose value was found to be 0.316 and 1.053 respectively as shown in the Table 5.

Robustness

When λ_{max} was varied within a limit of ± 2.0 nm, the % recovery data obtained lied in

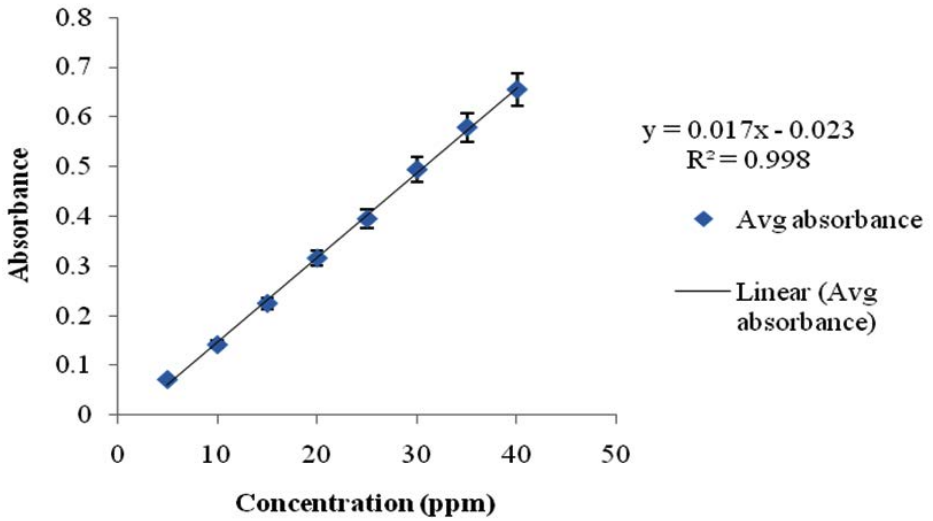

Fig. 5. Calibration curve of RT in SNF at 263 nm

Table 3. Inter-Day Precision Studies

Run#	Inter-Day Precision Studies					
	Low QC of RT, 5 µg/ml		Medium QC of RT, 30µg/ml		High QC of RT, 60 µg/ml	
	Observed Conc.	% Relative Error	Observed Conc.	% Relative Error	Observed Conc.	% Relative Error
SET 1						
RUN 1	5.02	0.4	30.14	0.47	60.12	0.200
RUN 2	4.96	-0.8	29.69	-1.03	60.96	1.600
RUN 3	5.05	1	30.11	0.37	61.42	2.367
MEAN	5.01	0.2	29.98		60.833	1.389
SD	0.045826		0.205		0.538	-99.103
%RSD	0.914686		0.685		0.884758	-98.525
RUN 1	4.99	-0.2	30.25	0.83	60.59	0.983
RUN#2	5.11	2.2	29.55	-1.50	59.95	-0.083
RUN#3	5.12	2.4	29.86	-0.47	59.25	-1.250
MEAN	5.073333		29.887		59.930	
SD	0.072342		0.351		0.670	
%RSD	1.425922		1.174		1.118	
RUN#1	4.99	-0.2	30.49	1.63	60.16	0.267
RUN#2	5.11	2.2	30.99	3.30	60.66	1.100
RUN#3	5.09	1.8	31.4	4.67	59.25	-1.250
MEAN	5.063333		30.96		60.023	
SD	0.064291		0.46		0.715	
%RSD	1.269737		1.4720322		1.191	

Table 4. Intra-Day Precision Studies

Run#	Inter-Day Precision Studies					
	Low QC of RT, 5 µg/ml		Medium QC of RT, 30µg/ml		High QC of RT, 60 µg/ml	
	Observed Conc.	% Relative Error	Observed Conc.	% Relative Error	Observed Conc.	% Relative Error
SET 1						
RUN#1	5.02	0.4	30.29	0.97	60.32	0.533
RUN#2	4.98	- 0.4	29.87	- 0.43	60.45	0.750
RUN#3	5.09	1.8	30.15	0.50	61.02	1.700
MEAN	5.03	0.6	30.1033		60.597	0.994
SD	0.05567764		0.175		0.304	-99.493
%RSD	1.1069114		0.580		0.50169	-99.164
RUN#1	5.01	0.2	30.35	1.17	59.89	-0.183
RUN#2	5.11	2.2	29.48	-1.73	59.65	-0.583
RUN#3	5.02	0.4	29.99	-0.03	60.79	1.317
MEAN	5.05		29.940		60.110	
SD	0.05507571		0.437		0.601	
%RSD	1.09132838		1.460		1.000	
RUN#1	4.99	-0.2	29.89	-0.37	61.16	1.933
RUN#2	5.03	0.6	30.29	0.97	60.26	0.433
RUN#3	5.55	11	30.15	0.50	59.55	-0.750
MEAN	100.2567		30.11		60.323	
SD	0.261024		0.20		0.807	
%RSD	0.260355		0.67412		1.338	

Table 5. Values of LOD and LOQ for RT

Conc(µg/ml)	Absorbance (Avg)	Std. Dev	LOD	LOQ
5	0.072	0.002	0.353	1.176
10	0.142	0.0013	0.229	0.765
15	0.225	0.003	0.529	1.765
20	0.316	0.0011	0.194	0.647
25	0.395	0.0021	0.371	1.235
30	0.494	0.001	0.176	0.588
35	0.569	0.00258	0.455	1.518
40	0.623	0.00211	0.372	1.241
45	0.674	0.0011	0.194	0.647
50	0.715	0.0022	0.388	1.294
55	0.775	0.0019	0.335	1.118
60	0.835	0.0011	0.194	0.647
Average			0.316	1.053

Table 6. Results of Robustness

S.No	Conc(µg/ml)	λ_{max}	Analyzed Conc.	Abs(Avg.)	Std.Dev	% RSD	% Assay
1	30	261	30.118	0.489	0.013	0.0432	100.392
2	30	263	30.235	0.491	0.015	0.0498	100.784
3	30	265	29.941	0.486	0.011	0.0365	99.804

Table 7. Repeatability Result for RT-test solutions

Conc (µg/ml)	Avg. conc. (n=6)	Std.dev	% RSD
30	30.113	0.0015	0.004981

between 99.804 to 100.784 with a ± 2% confidence interval. The results of the robustness data are shown in Table 6.

Repeatability

The repeatability of the proposed method was evaluated by analyzing 30 µg/ml RT-test solution for a set of six times. The value of % RSD was found to be < 2 as shown in Table 7.

Summary

The validation parameters evaluated as per the proposed methods are found within the chosen range. The summary is shown in Table 8.

Table 8. Results of validated parameters

Validation parameter	Results
Absorption maxima (nm)	263 nm
Regression equation	0.017x-0.023
Linearity range (µg/ml)	5-60 µg/ml
Slope (m)	0.017
Intercept (c)	0.023
Value of R^2	0.998
% Recovery	100.25 %
Inter-day	0.685-1.472
Intra-day	0.260 -1.338
Value of LOD	0.316
Value of LOQ	1.053

CONCLUSION

The created UV spectrophotometric strategy is exact, straightforward, fast, exact, solid, delicate, reproducible, and prudent for the assurance of RT and its drug tablet dose structures. The reagents used in the proposed strategies are monetary, promptly accessible and the methodology doesn't include any basic response conditions or dreary example readiness. The techniques are more specific than a significant number of the revealed spectrophotometric strategies and utilize higher frequencies to gauge absorbance readings where the blunders because of idle fixings are limited generally. The strategies are liberated from obstructions from the normal excipients. The measurable boundaries and the

recuperation information uncover great exactness and accuracy of the techniques. These strategies can be utilized as broad techniques for the assurance of RT in mass powder and measurements structures. The techniques enjoy numerous upper hands over the division procedures like HPLC and incorporate diminished expense, and speed with high exactness. Subsequently, the strategies can be utilized in routine examination of medications in quality control labs.

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Conflict of Interest

There was no conflict of interest stated by authors.

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