EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 2
351-358
Year: 2018

DEVELOPMENT AND VALIDATION FOR ESTIMATION OF RIVASTIGMINE BY USING RP-HPLC AND UV-SPECTROPHOTOMETRY

Roopa Chirala* and Saisindhu Gande[2]

M.Pharm, 2nd Year, Department of Pharmaceutical Analysis & Quality Assurance, RBVRR Women's College of Pharmacy, Osmania University, Hyderabad, Telangana, India.

***Corresponding Author: Roopa Chirala**
M.Pharm, 2nd Year, Department of Pharmaceutical Analysis & Quality Assurance, RBVRR Women's College of Pharmacy, Osmania University, Hyderabad, Telangana, India.

Article Received on 12/12/2017 **Article Revised on 02/01/2018** **Article Accepted on 22/01/2018**

ABSTRACT
A simple, precise and accurate RP-HPLC and UV-Spectrophotometric method was developed and validated for determination of Rivastigmine in capsule dosage form. Chromatography was carried out by using binary isocratic elution at a flow rate of 1ml/min was employed on a symmetry Phenomenex C18 (250x4.6mm, 5µm in particle size) column at ambient temperature. The mobile phase consisted of Methanol: (0.01M) Ammonium formate (pH 4.0) at the ratio of 50:50 and 20µl sample was injected. The retention time for Rivastigmine was 3.80 min. Calibration curve was plotted with a range of 1-64µg/ml with correlation coefficient of 0.999 for RP-HPLC method. UV-Spectrophotometry method was developed and validated by using the same mobile phase. Calibration curve was plotted with a range of 5-30µg/ml with correlation coefficient of 0.998. The assay was validated in terms of linearity, precision, accuracy, specificity, limit of detection, limit of quantification and robustness. The percentage RSD values for all parameters were found to be less than 2% for RP-HPLC and UV-Spectrophotometric method. The proposed method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Rivastigmine in capsule dosage form and standard drug.

KEYWORDS: Rivastigmine, RP-HPLC, UV-Spectrophotometry, Methanol, Ammonium formate, ICH guidelines.

1. INTRODUCTION
Rivastigmine is chemically a carbamate derivative, reversible inhibitor of acetyl-cholinesterase used for the treatment of mild to moderate Alzheimer's disease which is associated with parkinson's disease. Rivastigmine is (S)-3-(1-(Dimethylamino) ethyl) phenol N-ethyl-N-methylcarbamate. Its empirical formula is $C_{14}H_{22}N_2O_2.2C_4H_6O_6$ having molecular weight of 250.33g/mol.

Literature survey reveals few spectrophotometric and chromatographic methods had been reported i.e., UV-Spectrophotometric, Visible Spectrophotometric, RP-HPLC methods and stability studies. Literature survey does not reveal any RP-HPLC and UV-spectrophotmetric method with this specific method. The purpose of developing and validating a method using a simple, rapid, sensitive, precise, accurate and specific RP-HPLC and UV-Spectrophotometry method is the retention time and the run time for this method was very short, hence it requires less mobile phase for these method, making it more economical and rapid. This method can be used for analysis of large number of samples.

2. MATERIALS AND METHODS
2.1. Chemicals and Reagents
Pure standard drug was procured from Orbit Scientific co., Ltd. The capsule formulation with label claim of 3mg (RIVAMER) was purchased from Sai Medical Hall, Hyderabad, India.

Methanol, Water, Acetonitrile, Ammonium formate was procured from SD Fine Chem Limited. The formic acid was procured from SRL Limited.

2.2. Instrumentation
The analysis was performed by using Schimazdu 20AD RP-HPLC instrument with Phenomenex Luna C_{18} column (250x4.6 mm) 5µm, it contains Rhenodyne valve with 20µl fixed loop injector with UV- Visible detector. The UV-Spectrophotometry instrument i.e., Elico SL 210 with Spectra Treats software, analytical balance (Contech) is used for weighing, pH (Elico), Sonicator (Labotech) was used for degassing the mobile phase.

2.3. Chromatographic Conditions
The RP-HPLC analysis was carried out on a C_{18} Phenomenex (250x4.6mm, 5µm particule size) column. The mobile phase consists of a mixture of (0.01M)

Ammonium formate at pH 4.0 (adjusted with formic acid): Methanol (50:50v/v). The flow rate was 1ml/min with isocratic elution and the detection was carried out at 217nm. The injection volume is 20µl and the run time was 10mins.

2.4. Preparation of 0.01M Ammonium Formate buffer

Dissolve 0.63gms of Ammonium formate in 750ml water (Hplc) and adjust the pH of the buffer with formic acid to pH 4.0 and make up to 1000ml with water.

2.5. Preparation of Mobile Phase

Mix 50ml of pH-4.0 (0.01M) Ammonium formate buffer and 50ml of Methanol and degas it.

2.6. Preparation of Rivastigmine Standard Stock Solution

Accurately weigh 10mg of Rivastigmine-L-Tartrate and transfer it in a clean dry 10ml volumetric flask and 7ml of diluent was added, shake well until it get dissolved and volume was made up to 10ml with the same diluent (i.e., 1000µg/ml).

Further pipette out 1ml from the above solution in 10ml volumetric flask and make up to mark with the diluent (i.e., 100µg/ml).

2.7. Preparation of Rivastigmine Sample Solution

Accurately weigh 20 capsules and average weight was calculated. The capsule powder equivalent to 10mg (i.e., 0.710gm) of Rivastigmine was weigh and transferred in to 10ml volumetric flask. To that 7ml of diluent was added and sonicated until it gets dissolved and make up to mark with the diluent. Filter through 0.45µ filter (1000µg/ml).

Further pipette out 1ml from the above solution in 10ml volumetric flask and make up to mark with diluent (i.e., 100µg/ml).

2.8. Selection of detection Wavelength

The 10µg/ml solution was scanned in the wavelength range of 200-400nm in order to observe maximum absorbance. The λ_{max} for Rivastigmine-L-Tartrate was found to be 217nm, since it shows the maximum absorbance at that particular wavelength.

2.9. Method Validation

Both the RP-HPLC and UV-Spectrophotometric method were developed and validated by considering the parameters such as linearity, precision, accuracy, robustness, specificity, LOD and LOQ.

System Suitability

In RP-HPLC, it is the integral part of the method development which is used to ensure the performance of the RP-HPLC system. The parameters such as retention time (R_t), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicates injections at a concentration of 20µg/ml.

Linearity

Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

Accuracy

It is the measure of the closeness of the experimental value to the true value. Accuracy should be established across the specified range of the analytical procedure.

Precision

The precision of an analytical procedure expresses the closeness of a agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

Intra Day Precision

It expresses the precision under the same operating conditions over a short interval of time.

Inter Day Precision

It expresses the precision between laboratories variations, different days, different analysts etc.

Limit of Detection

It is the lowest concentration of the analyte in a sample which can be detected but not necessarily quantified, as an exact value under the stated.

Limit of Quantification

It is the lowest concentration of the analyte in a sample which can be detected and quantified.

Robustness

It is a measure of the method capability to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

3. RESULTS AND DISCUSSION

3.1. Results of UV-Spectrophotometry Method

Linearity

The linearity of UV-Spectrophotometric method was found to be in the concentration range of 5-30µg/ml at 217nm with the correlation coefficient of 0.998. The statistically obtained results were given in table-1.

Table-1: Results of linearity.

CONCENTRATION (µg/ml)	ABSORBANCE
5	0.2967
10	0.4585
15	0.5619
20	0.6998
25	0.8277
30	0.9657

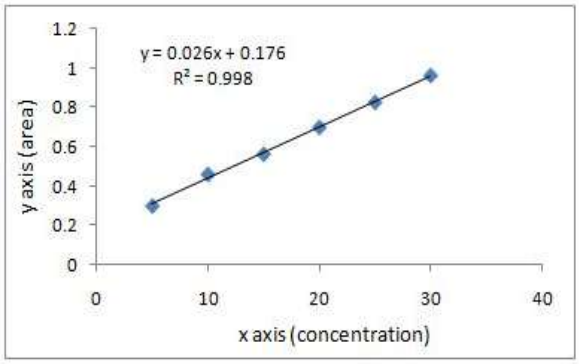

Fig. 1: Linearity Graph by UV-Spectrophotometry.

Accuracy

The accuracy was developed by recovery studies which were carried out at three different spiked levels i.e., 80%, 100%, 120%. The statistically obtained results are given in table-2.

Table-2: Results of accuracy.

Spike level (%)	Concentration(µg/ml)		Total concentration (µg/ml)	Absorbance	% Recovery	Avg % Recovery	%RSD
	Formulation (µg/ml)	Pure drug (µg/ml)					
80	7.5	6.0	13.5(µg/ml)	0.5264	99.83	99.83	0.5129
100	7.5	7.5	15.0(µg/ml)	0.5619	98.95	99.74	0.4819
120	7.5	9.0	16.5(µg/ml)	0.6085	100.82	99.82	0.7574

Precision

The precision was carried out by the 15µg/ml concentration and the statistically obtained results are given in table-3 & 4.

Table-3: Results of Intra Day Precision.

Concentration (µg/ml)	Absorbance	%Assay
15µg/ml	0.5621	99.00
15µg/ml	0.5636	99.38
15µg/ml	0.5595	98.33
15µg/ml	0.5644	99.59
15µg/ml	0.5656	99.90
15µg/ml	0.5642	99.54
Mean	0.563	
S.D	0.002	
%RSD	**0.383**	

Table-4: Results of Inter Day Precision.

Concentration (µg/ml)	Absorbance	%Assay
15µg/ml	0.5675	100.38
15µg/ml	0.5642	99.54
15µg/ml	0.5685	100.64
15µg/ml	0.5708	101.23
15µg/ml	0.5695	100.90
15µg/ml	0.5702	101.08
Mean	0.568	
S.D	0.002	
%RSD	**0.421**	

LOD & LOQ

The LOD and LOQ was performed using UV-Spectrophotometry the Statistical results obtained are given in table-5.

Table-5: Results of LOD & LOQ.

Parameters	Slope	Intercept
1	0.025	0.181
2	0.026	0.176
3	0.026	0.157
Mean	**0.0257**	0.1713
SD	-	**0.0127**
LOD	(3.3) x SD. of interception/mean o f slope	(3.3) x 0.0127/0.0257 = **1.6280 µg/ml**
LOQ	(10) x SD. of interception/mean o f slope	(10) x 0.0127 /0.0257 =**4.9334 µg/ml**

Assay of marketed formulation

By performing the assay of marketed formulation RIVAMER 3mg (capsule) the statistical results obtained are given in table-6.

Table-6: Results of Assay.

PARAMETERS	VALUES OBTAINED
Dosage form	Capsule (oral)
Labelled claim	3mg
Amount found	2.97mg
% Recovery	98.91%
%RSD	0.369%

3.2. Results of RP-HPLC Method

System Suitability

The six replicates of 20µg/ml concentration were injected and the Statistical results obtained are given in table-7.

Table-7: Results of system suitability.

S.no	Retention Time	Area	Theoretical plates	Tailing factor
1	3.797	419610	4582	1.38
2	3.839	420276	4543	1.39
3	3.816	420771	4567	1.40
4	3.817	420877	4536	1.38
5	3.824	421590	4567	1.38
6	3.806	422004	3930	1.69
Mean	3.819	420624	**4559**	**1.386**
SD	0.015	736.21	18.98	0.008
%RSD	**0.397**	**0.175**	0.416	0.645
Limits	NMT 1.0	NMT 2.0	NLT 2000	NMT 2.0

Specificity

By performing the specificity by RP-HPLC method the obtained chromatograms are given below in fig: 2-4.

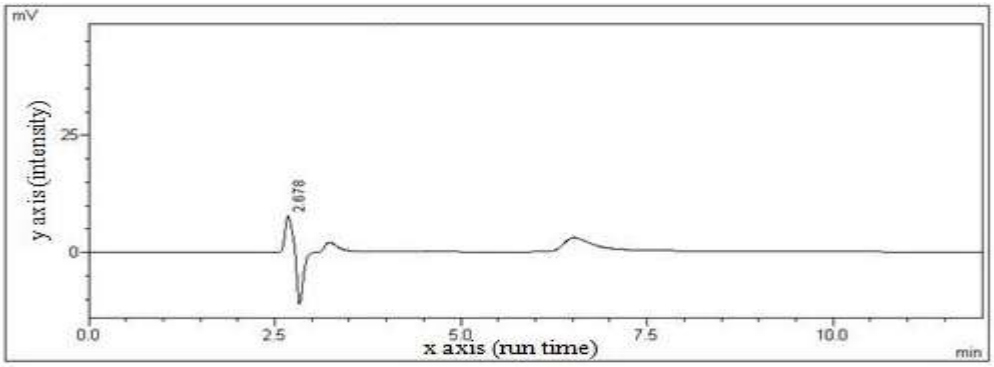

Figure-2: Blank Chromatogram.

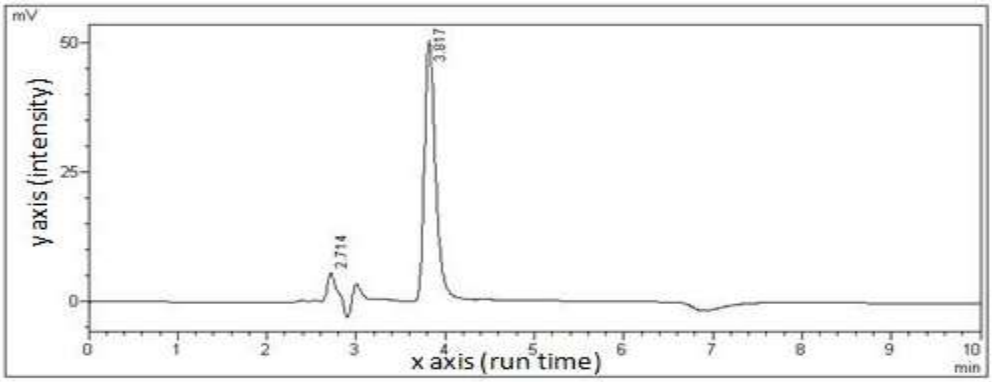

Figure-3: Standard Chromatogram of Rivastigmine (20µg/ml).

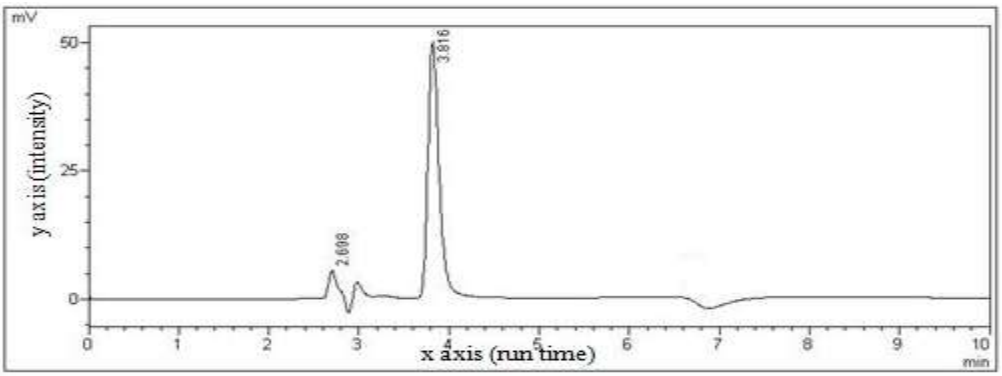

Figure -4: Sample Chromatogram of Rivastigmine (20µg/ml).

Linearity

The linearity of RP-HPLC method was found to be in the concentration range of 1-64µg/ml at 217nm with correlation coefficient of 0.999. The statistically obtained results were given in table-8, the standard chromatogram obtained is given in fig-5 and the plot so obtained was given in fig-6.

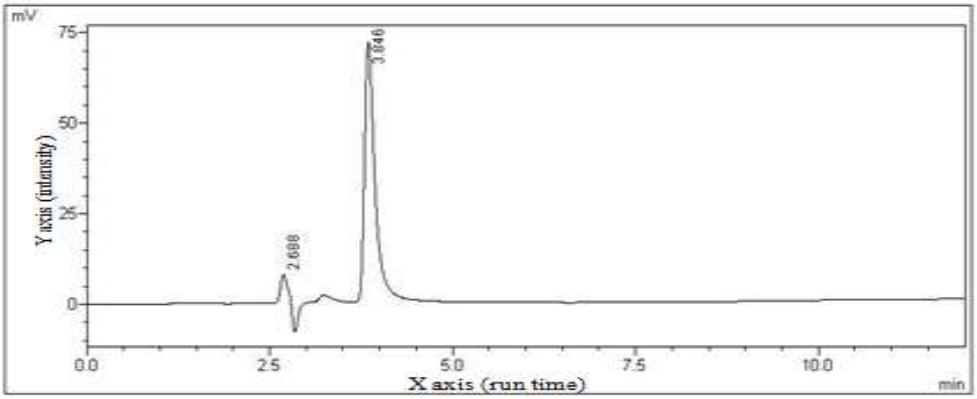

Fig-5: Standard Chromatogram of Rivastigmine (32µg/ml).

Table-8: Results of linearity.

CONCENTRATION (µg/ml)	AREA
1	33505
2	41755
4	88897
8	171541
16	354162
20	419610
32	661785
64	1351407

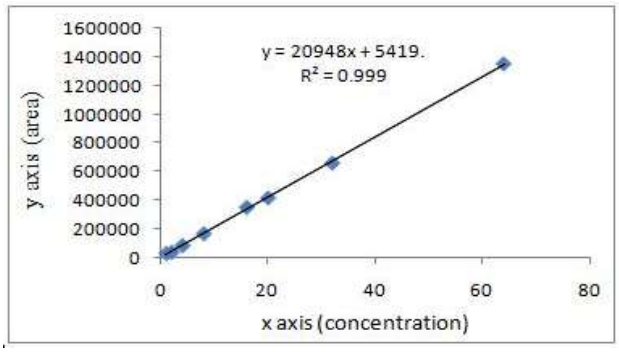

Fig- Linearity Graph by RP-HPLC.

Accuracy

The accuracy was developed by recovery studies which were carried out at three different spiked levels i.e., 80%, 100% and 120%. The statistically obtained results are given in table-9.

Table-9: Results of accuracy.

Spike level (%)	Concentration (µg/ml)		Total Concentration (µg/ml)	Area	% Recovery	Avg % Recovery	%RSD
	Formulation	Pure drug					
80%	10	8	18 µg/ml	377169	99.3	99.6	0.3857
100%	10	10	20 µg/ml	418761	99.2	99.6	0.3265
120%	10	12	22 µg/ml	465735	100.3	99.8	0.4388

Precision

The precision was carried out by 20µg/ml concentration and the statistical results which had obtained are given in table-10&11.

Table-10: Results of Intra Day Precision.

S.NO	Retention Time	Area
1	3.824	422926
2	3.801	423090
3	3.817	422238
4	3.817	423678
5	3.806	422741
6	3.811	423588
Mean	3.813	422934
S.D	0.009	524.308
%RSD	0.243	0.124

Table-11: Results of Inter Day Precision.

S.NO.	Retention Time	Area	%Assay
1	3.801	424170	100.87
2	3.839	424060	100.85
3	3.817	424976	101.07
4	3.824	425328	101.15
5	3.816	423382	100.68
6	3.811	425556	101.21
Mean	424578		
S.D	841.735		
%RSD	0.1982		

Robustness

The robustness was performed by changing the flow rate (1ml/min±0.1ml/min), by changing the pH (4.0±0.2) and by changing the mobile phase ratio (50:50±2). The results obtained are given in table: 12-14.

Table-12: Results of change in flow rate (1ml/min±0.1ml/min).

PARAMETERS	FLOW-0.9ml/min		FLOW-1.1ml/min	
S.NO.	Retention Time	Area(x)	Retention Time	Area(x)
1	3.938	428265	3.797	421631
2	3.939	422844	3.707	426747
3	3.915	425303	3.799	427568
Mean	425470.7		425315.3	
S.D	2714.3		3217.02	
%RSD	0.637%		0.756%	

Table-13: Results of change in pH (4.0±0.2).

PARAMETERS	pH-4.2		pH-3.8	
S.NO.	Retention Time	Area(x)	Retention Time	Area(x)
1	3.799	425565	3.938	425309
2	3.797	420359	3.915	426431
3	3.797	423166	3.939	422844
Mean	423030		424861	
S.D	2605.6		1834.9	
%RSD	0.615%		0.431%	

Table-14: Results of change in Mobile Phase (50:50±2).

PARAMETERS	RATIO-BUFFER: METHANOL (48:52)		RATIO-BUFFER: METHANOL (52:48)	
S.No.	Retention Time	Area(X)	Retention Time	Area(X)
1	3.797	419610	3.938	426781
2	3.707	423565	3.939	424342
3	3.797	421023	3.945	419912
Mean	421399		423678	
S.D	2004.1		3482.2	
%RSD	0.475%		0.821%	

LOD & LOQ

The LOD & LOQ was performed using RP-HPLC the statistical results obtained are given in table-15.

Table-15: Results of LOD & LOQ.

Parameters	Slope	Intercept
1	21168	2863
2	20948	5419
3	21020	4572
Mean	21045.33	4284.67
SD	-	1302
LOD	(3.3) x sd of interception/mean of slope	(3.3) x 1302/21045.33 = **0.20 µg/ml**
LOQ	(10) x sd of interception/mean of slope	(10) x 1302/21045.33 = **0.61 µg/ml**

Assay of Marketed formulation

By performing the assay of marketed formulation RIVAMER 3mg (capsule) the statistical results obtained are given in table-16.

Table-16: Results of Assay.

PARAMETERS	VALUES OBTAINED
Dosage form	Capsule (oral)
Labeled claim	3mg
Amount found	2.97mg
% Recovery	98.95%
%RSD	0.371%

CONCLUSION

The developed RP-HPLC and UV-Spectrophotometric method for estimation of Rivastigmine-L-Tartrate in bulk and pharmaceutical dosage form was found to be simple, specific, accurate, precise, linear and robust. Compared to UV-Spectrophotometric method the RP-HPLC method was found to be more sensitive.

It can be concluded that the developed method was a good approach for obtaining results and were found to be a suitable for routine estimation of Rivastigmine-L-Tartrate in pharmaceutical formulations.

ACKNOWLEDGEMENT

The authors would like to the management of RBVRR women's college of pharmacy (RBVRRWCP), for providing the necessary facilities to carry out this research work.

REFERENCES

1. Rang H.P., Ritter J.M., Flower R.J., Henderson; Rang and Dale's pharmacology; an international 8th edition, 2016; 482-496.
2. Vaidehi Jha N., A.K. Seth, Sharad Kumar, Yogesh Chand Yadav, 2011; Development of a rapid, accurate and sensitive uv spectroscopic method for estimation of rivastigmine tartrate entrapped in nanoparticles; *Pharma science monitor(an international journal of pharmaceutical sciences)*, 2(3).
3. Vessman J. 1996; Validation of analytical methods from the perspective of an analytical chemist in the pharmaceutical industry; *journal of Pharm & biomed analysis*, 14: 867–869.
4. Winslow P.A., defining a master plan for the validation of analytical methods, j. Validation technology, Instrumentation of the RP-HPLC & method development & design of separation & system suitability parameters, 1997; 361-367.
5. www.chem.agilent.com/cag/cabu/buffersel.htm
6. www.chromacademy.com/Buffer-Choice-for-HPLC-separations.html
7. www.drugs.com/monograph/rivastigmine.html
8. www.fda.gov/downloads/drugs/guidancecompliance regulatoryinformation/guidances/ucm201278.pdf