Research Article

# A Novel RP-HPLC and Visible Spectrophotometric Methods for the Quantification of Rivastigmine in Bulk and Pharmaceutical Formulations

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**ABSTRACT**

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Rivastigmine in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Water: Acetonitrile (ACN) 35:25:40 v/v, ($P^H$ 4.8). The UV detection wavelength was 211 nm and 20µl sample was injected. The retention time for Rivastigminewas 4.60 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Rivastigminein tablet dosage form and bulk drug.

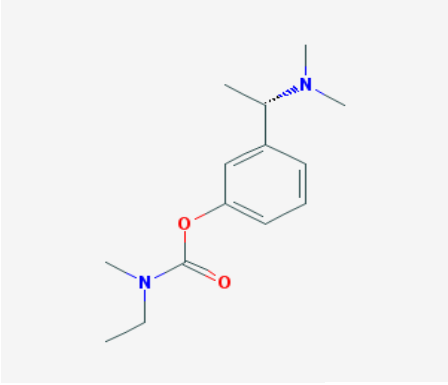
**Keywords:** Rivastigmine, RP-HPLC, UV detection, recovery, precise, 211nm.

## INTRODUCTION

## DRUG PROFILE

IUPAC Name : 3-[(1S)-1-(dimethylamino) ethyl] phenyl N-ethyl-N-methylcarbamate

**CHEMICAL STRUCTURE**



Molecular formula : $C_{14}H_{22}N_2O_2$

Molecular weight : 250.3367

Physical appearance : white crystalline powder

Category :Parasympathomimetics, neuroprotective agents, cholinesterase inhibitors , cholinergic agents

Solubility : freely soluble in water and methanol

Mechanism of action : It exert its effect by enhancing cholinergic function

pH of the solution : 4.8

Routes : Oral, transdermal

Excretion : Renal, 97%

Trade name : Exelon

## EXPERIMENTAL

### Materials

Working standard of Rivastigmine was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

### Apparatus

A Series HPLC system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was

used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

**Determination of wavelength of maximum absorbance**

The standard solutions of Rivastigmine were scanned in the range of 200 -400 nm against mobile phase as a blank. Rivastigmine showed maximum absorbance at 211nm. So the wavelength selected for the determination of Rivastigminewas 211nm.

**Chromatographic equipment and conditions**

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of RIVASTIGMINE an isocratic PEAKHPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. Theinstrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol: Water: Acetonitrile(ACN) 35:25:40,($P^H$ 4.8)Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 211nm with 10min runtime.

**Standard and sample solutions**

A 10 mg amount of Rivastigmine reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20Sandoz (capsule 6mg) capsules was prepared by grinding them to a fine, uniform size powder. 10 mg of Rivastigmine was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase was added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 80ppm.

**Method validation**

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

**System Suitability**

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

**Table 1: System suitability parameters of Rivastigmine**

| | |
|---|---|
| Api Concentration | 80ppm |
| Mobile Phase | Methanol: Water: Acetonitrile(ACN) |
| Wavelength | 211nm |
| Column | $C_{18}$ Column |
| $P^H$ | 4.8 |
| Concentration | 80ppm |
| Retention Time | 4.60min |
| Run Time | 10min |
| Area | 240056 |
| Th. Plates | 9814 |
| Tailing Factor | 1.32 |
| Pump Pressure | 10.5 MPa |

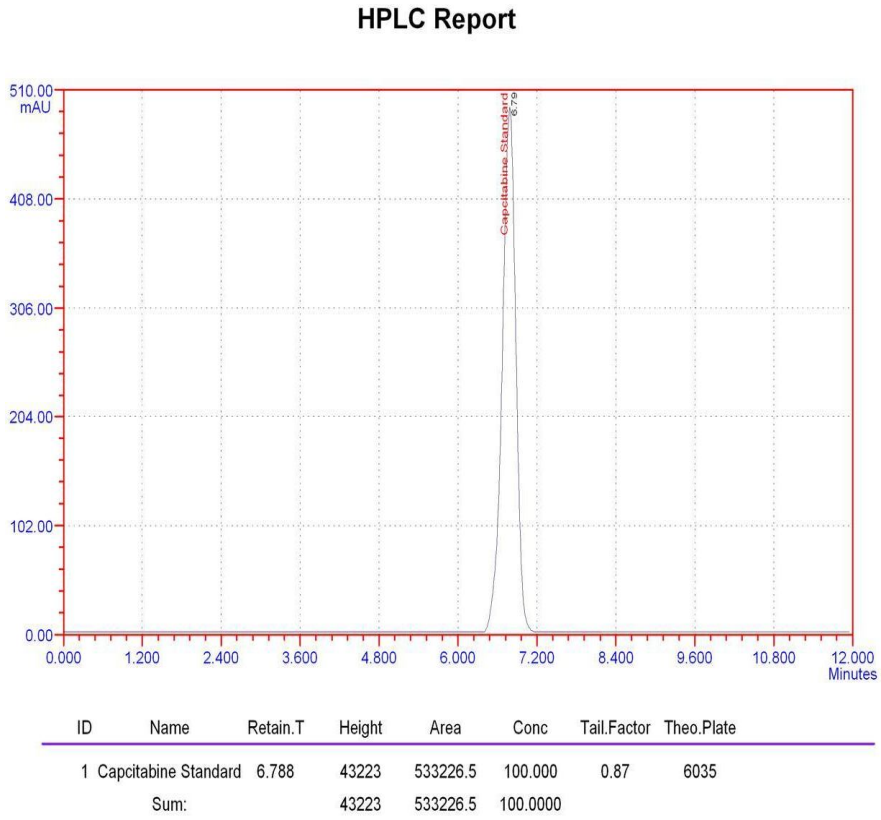



**Fig. 2: Standard chromatogram of Rivastigmine**

**Range of linearity**

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 20,40,60,80,100 and 120ppm for Rivastigmine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y =4114+3084(r= 0.999). Linearity values can shown in Table: 2

**Table 2: Linearity results of Rivastigmine**

| S.No | Concentration (ppm) | Area |
|---|---|---|
| 1 | 20 | 61268 |
| 2 | 40 | 110648 |
| 3 | 60 | 179396 |
| 4 | 80 | 240056 |
| 5 | 100 | 305209 |
| 6 | 120 | 369930 |
| | Slope<br>Intercept<br>CC | 3084.071<br>4114.71<br>0.99939 |

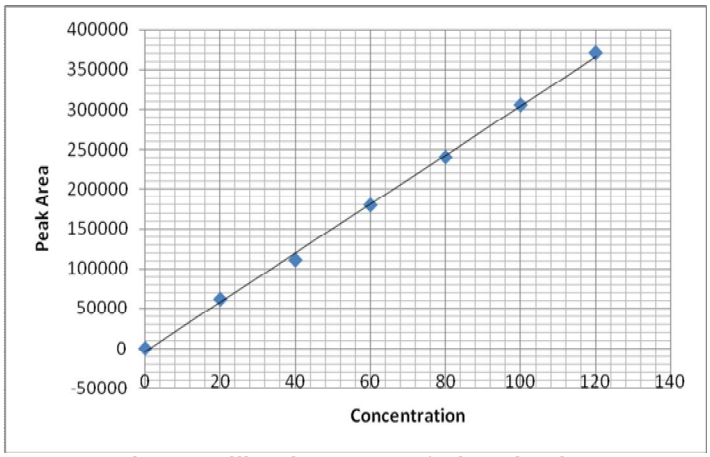
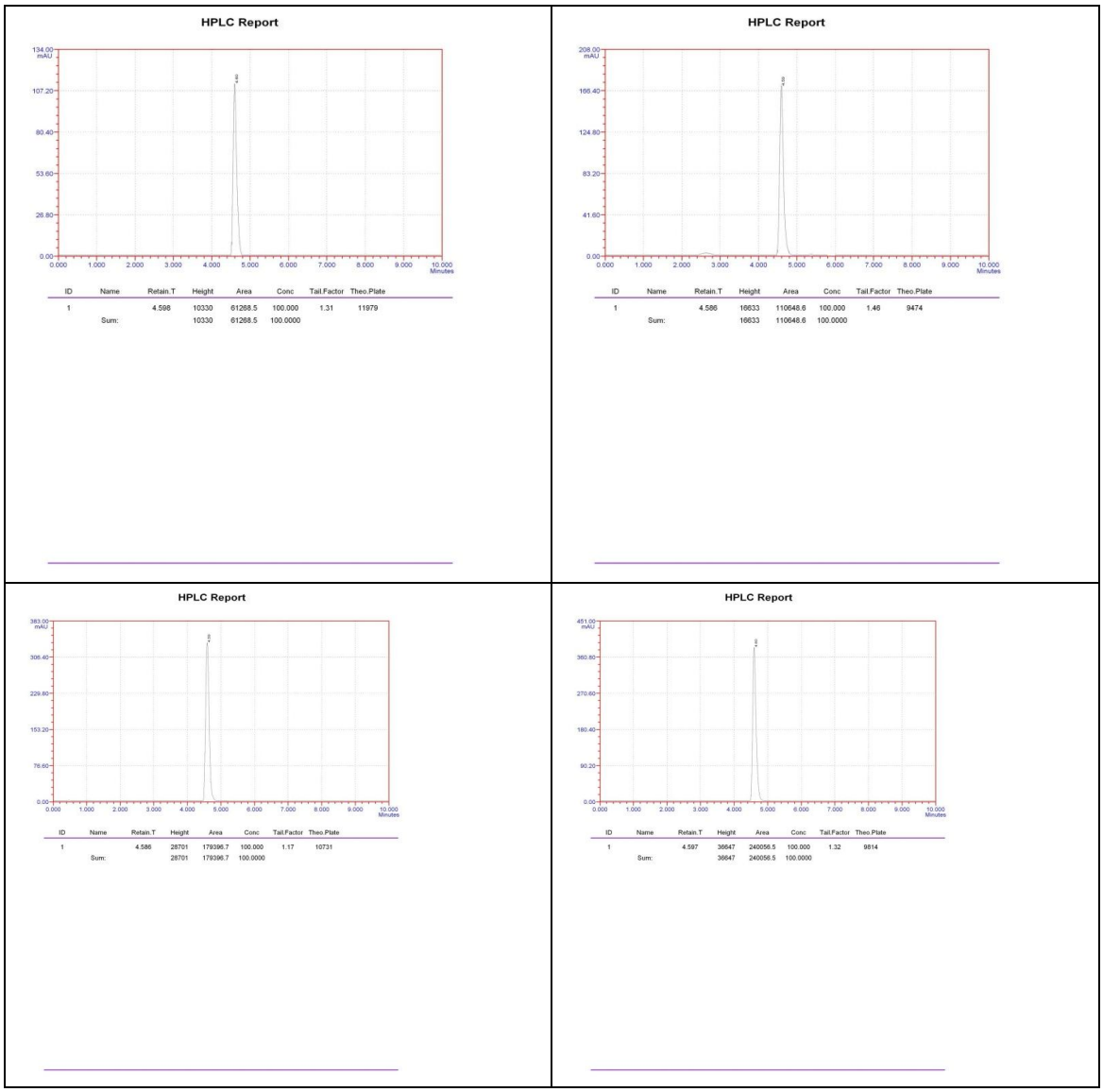
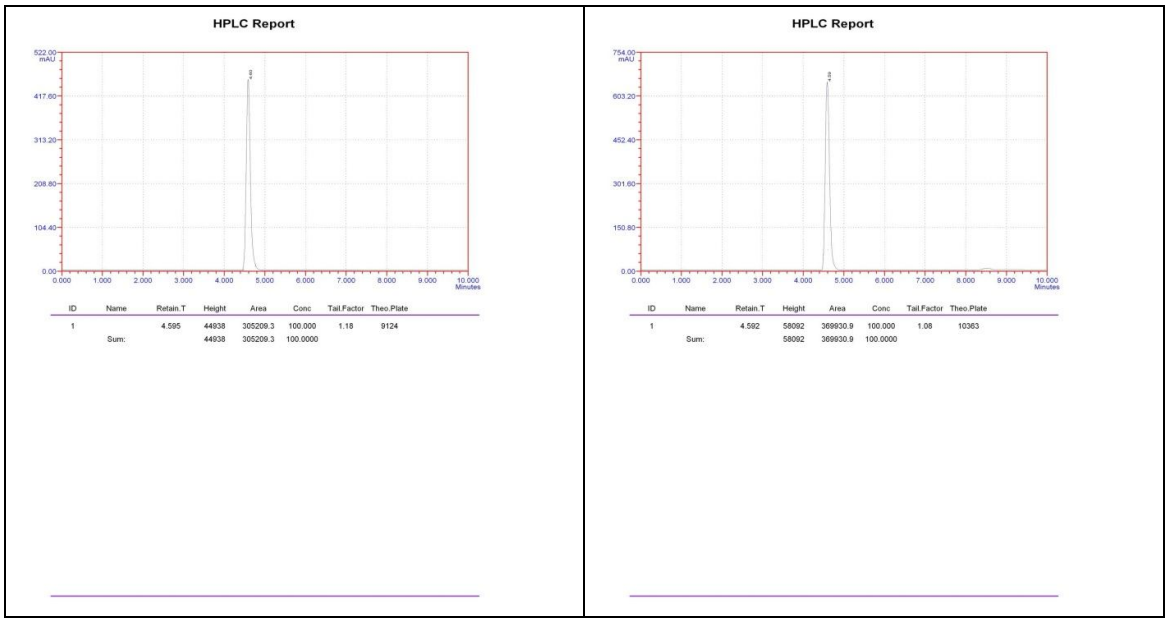



**Fig. 3: Calibration curve of Rivastigmine**






**Precision**

To study precision, six replicate standard solutions of Rivastigmine(80ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

**Table 3: Intraday Precision Results for Rivastigmine**

| Sample (µg/ml) | Area |
|---|---|
| 1 | 240056 |
| 2 | 240859 |
| 3 | 240245 |
| 4 | 230540 |
| 5 | 239193 |
| 6 | 239490 |
| **RSD** | **1.63** |

**Table 4: Inter day Precision results of Rivastigmine**

| **Sample (µg/ml)** | **Area** |
|---|---|
| 1 | 233697 |
| 2 | 235602 |
| 3 | 231387 |
| 4 | 233544 |
| 5 | 228735 |
| 6 | 224212 |
| **RSD** | **1.79** |

**Limit of Detection and Limit of Quantification**

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 2ppm dilution Peak was not clearly observed, based on which 2ppm is considered as Limit of Detection and Limit of Quantification is 6.6ppm.

**Table 5: LOD and LOQ results of Rivastigmine**

| **Parameter** | **Measured Value** |
|---|---|
| Limit of Quantification | 6.6ppm |
| Limit of Detection | 2ppm |

**Robustness**

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Rivastigmine at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 6.

**Table 6: Robustness results of Rivastigmine**

| S.NO | Parameter | Change | Area | % of Change |
|---|---|---|---|---|
| 1 | Standard | …… | 240056 | …… |
| 2 | MP | Methanol:water:ACN<br>30:25:45<br>40:25:35 | 240112<br>241323 | 0.02<br>0.52 |
| 3 | PH | 4.9<br>4.7 | 242904<br>243222 | 1.18<br>1.01 |
| 4 | WL | 214nm<br>208nm | 241073<br>243045 | 0.42<br>1.24 |

**Ruggedness**

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

| Sample (µg/ml) | Area |
|---|---|
| 1 | 229494 |
| 2 | 226720 |
| 3 | 221288 |
| 4 | 225010 |
| 5 | 229709 |
| 6 | 221407 |
| **RSD** | **1.65** |

**Recovery**

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 60ppm, 80ppm, 100ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 99.2 to 100.8 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.8

**Table 8: Recovery results of Rivastigmine**

| % Recovery | Target Conc., (ppm) | Spiked conc, (ppm) | Final Conc, (ppm) | Conc., Obtained | % of Recovery |
|---|---|---|---|---|---|
| 50% | 40 | 20 | 60 | 59.9 | 99.9 |
| | 40 | 20 | 60 | 59.5 | 99.2 |
| | 40 | 20 | 60 | 60.08 | 100.1 |
| 100% | 40 | 40 | 80 | 80.4 | 100.5 |
| | 40 | 40 | 80 | 80.6 | 100.7 |
| | 40 | 40 | 80 | 80.2 | 100.2 |
| 150% | 40 | 60 | 100 | 99.4 | 99.4 |
| | 40 | 60 | 100 | 100.8 | 100.8 |
| | 40 | 60 | 100 | 100.8 | 100.8 |

**Table 9: Formulation analysis**

| Formulation | Brand name | Prepared conc | Area | Amount found | %Assay |
|---|---|---|---|---|---|
| Rivastigmine | Sandoz (capsule 6mg) | 80ppm | 524878 | 78.95 | 98.4 |

**CONCLUSION**

The proposed method for the assay of Rivastigminein tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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