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A validated RP-HPLC method for estimation of Rivastigmine in pharmaceutical formulations

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ABSTRACT

An isocratic RP-HPLC Method for analysis of Rivastigmine in pharmaceutical dosage forms has been developed and validated. Best separation was achieved on a Thermo Hypersil C4 column (25 cm X 4.6 mm, 5 µm) using a mobile phase of 0.01 M ammonium acetate buffer adjusted to pH 4.0 with orthophosphoric acid and Acetonitrile (60:40, v/v) at a flow rate of 1.0 mL min-1. UV detection was performed at 220 nm. Atrovastatin was used as an internal standard. The retention time of Rivastigmine and Atrovastatin was 4.75 and 8.83 min, respectively. The method was validated for specificity, linearity, precision, accuracy, and limit of quantification, limit of detection, robustness, and solution stability. The proposed method was applied for the quantitative determination of Rivastigmine in commercial formulations.

Key Words: Rivastigmine, RP-HPLC, Method development.

INTRODUCTION

Dementia is a progressive brain dysfunction, which results in a restriction of daily activities and in most cases leads in the long term to the need for care. Alzheimer's disease (AD) is the most frequent type of dementia in old age [1]. People with Alzheimer's disease suffer mainly from impaired memory and orientation, limitations of concentration, planning and judgement, personality changes and later also perceptual, speech and walking disorders. Neuropath logically, Alzheimer's disease is characterized by the presence of neurofibrillary tangles and senile plaques, impaired synaptic function and cell loss [2]. Alzheimer's disease is recognized as being one of the most important challenges facing medicine in the 21st century due to aging population and high cost of managing the disease. Rivastigmine is chemically (−)*S*-*N*-ethyl-3-[(1-dimethyl-amino)ethyl]-*N*-methyl phenyl-carbamate hydrogen tartarate, a carbamate inhibitor of acetyl cholinesterase is used for the treatment of mild to moderate Alzheimer's disease in adults[3]. The

literature survey revealed that a few high performance liquid chromatography (HPLC) methods reported are applicable for analysis of Rivastigmine in body fluids [4-10]. A Spectroflurimetric [11] method has been developed for the determination of Rivastigmine in pharmaceuticals. There are no reports on the HPLC determination of Rivastigmine in pharmaceutical formulations. The present investigation describes precise, accurate and specific RP-HPLC method for the determination of Rivastigmine.

MATERIALS AND METHODS

Acetonitrile, Methanol HPLC grade were procured from E.merck (India) Ltd, Mumbai. Ammonium acetate and Orthophosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. A Reference standard of Rivastigmine was procured from Orchid Chemicals &Pharmaceuticals Ltd, Chennai.

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), UV detector, and Rheodyne 7725i injector with 50µl loop volume. A Thermo Hypersil C4 column (250 mm x 4.6 mm, 5 µm) was used for the separation.

Preparation of mobile phase and standard solutions:
Ammonium acetate buffer (0.01 M) adjusted to pH 4.0 with orthophosphoric acid and Acetonitrile (60:40, *v/v*). It was filtered through a 0.2 µ membrane filter and degassed. About 6 mg of Rivastigmine was accurately weighed and transferred to a 100ml volumetric flask and dissolved in mobile phase by sonication to give standard stock solution.

Preparation of sample solutions:
Twenty capsules were taken, average weight was determined and mixed well fine powder. Powder equivalent to average weight of 20 capsules was taken and dissolved in 100ml mobile phase and the Solution was filtered through 0.45µ membrane filter and then the filtrate was further diluted to get the required concentrations.

Assay method:
With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of Rivastigmine and Atrovastatin was found to be, 4.75 and 8.83 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated. The concentration of the drug calculated (Table 1) using following formula,

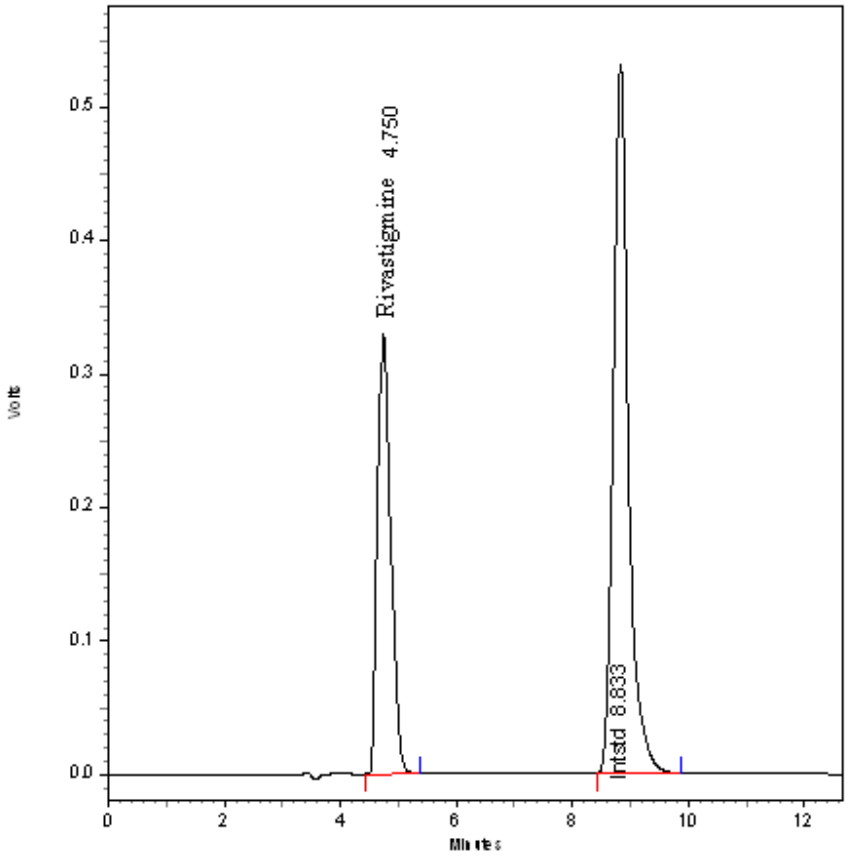
$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

RESULTS AND DISCUSSION

Estimation of Rivastigmine in dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. UV Detection was done at 220 nm. Typical chromatogram of sample solution is given in (Figure 1)**.** The peak area ratio of standard and sample solutions was calculated. The assay procedure was repeated for five times and mean peak area ratio and mean weight of standard drugs were calculated. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated and presented in (Table 1**.)** The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

The method was validated as per ICH guidelines. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in (Table 1). From the data obtained, added recoveries of standard drugs were found to be accurate.

Figure 1- Typical Chromatogram of Sample Solution of Rivastigmine



The linearity and range Rivastigmine was found to be from 1.5 to 7.5 µg/ml. (Table 2)**.** The calibration curve was constructed by plotting response factor against concentration of drugs. The

slope and intercept value for calibration curve was y = 0.0072 x -0.001 (R^2=0.998). The results show that an excellent correlation exists between response factor and concentration of drug within the concentration range indicated above. The calibration curve shown in (Figure 2).

Table 1- Results of analysis of formulation and recovery studies

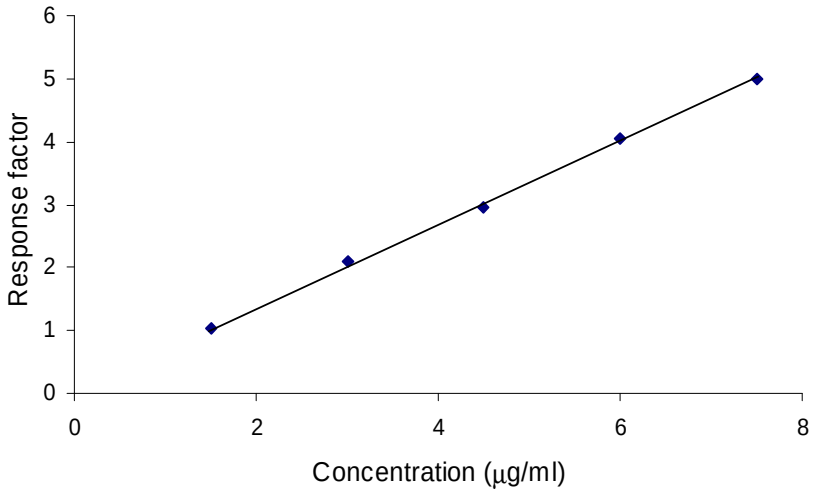
Drug	Amount mg/tab		% Label claim*	% Recovery
	Labelled	Found		
Rivastigmine	**6.0**	**5.83 ± 1.69**	**97.44 ± 1.53**	**98.03 ± 1.26**

* *Average of 6 determinations ± standard deviation*

Table 2- Linearity and range

Concentration (µg/ml)	Peak area
1.5	504733
3.0	1031468
4.5	1566001
6.0	2083000
7.5	2575820

Figure 2- Calibration curve of Rivastigmine



The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated**.** In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated, From the data obtained, the developed HPLC method was found to be precise.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Rivastigmine was found to be 5

ng/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ for Rivastigmine was found to be 15 ng/ml (Table 3)**.**

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Phenomenex LUNA C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed are rugged and robusted.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of Rivastigmine remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5hr, which was sufficient to complete the whole analytical process. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 3)**.** The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Table 3- Validation and system suitability studies

S. No.	Parameters	Rivastigmine
1	Linearity range	1.5 to 7.5 µg/ml
2	Regression equation Y = mx + c*	y = 0.0072 x -0.001
3	Correlation coefficient	0.9998
4	Theoretical plate/meter	12126
5	Resolution factor	3.32
6	LOD (ng/ml)	5
7	LOQ (ng/ml)	15

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of Rivastigmine in pharmaceutical dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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