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QUANTIFICATION METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF RIVASTIGMINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Rivastigmine in its pure form as well as in pharmaceutical dosage forms. Chromatography was carried out on Inertsil, C-18, 250 x 4.6 mm. 5 µ using a mixture of Phosphate buffer and Acetonitrile (70 : 30 v/v) as the mobile phase at a flow rate of 1.0 mL/min the detection was done by UV at 217nm. The retention time of the drug was 3.66 ± 0.25 min min. The method produced linear responses in the concentration range of 10-100 µg/mL of Rivastigmine. The method was found to be reproducible for analysis of the drug in tablet dosage forms.

Key words: Rivastigmine, RP-HPLC, Estimation.

INTRODUCTION

Rivastigmine, (*S*)-*N*-Ethyl-*N*-methyl-3-[1-(dimethylamino)ethyl]phenyl carbamate Rivastigmine is a carbamate derivative that is structurally related to physostigmine, but not to donepezil and tacrine. The precise mechanism of rivastigmine has not been fully determined, but it is suggested that rivastigmine binds reversibly with and inactivates chlolinesterase (eg. acetylcholinesterase, butyrylcholinesterase), preventing the hydrolysis of acetycholine, and thus leading to an increased concentration of acetylcholine at cholinergic synapses. The anticholinesterase activity of rivastigmine is relatively specific for brain acetylcholinesterase and butyrylcholinesterase compared with those in peripheral tissues. A literature survey revealed that only a few HPLC[1-5] methods are available for the estimation of Rivastigmine. The authors have proposed a new validated, sensitive and reproducible

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HPLC method for the determination of Rivastigmine and the dosage forms.

EXPERIMENTAL

Chromatographic conditions

A prominence isocratic HPLC system (Younglin HPLC YL9000 series) with YL 9110 Pump and with "autochro 3000" software and UV-Vis detector YL 9120, Inertsil C_{18} ODS column (250 x 4.6 mm, 5 µ) was used. A 20 µL Hamitton injection syringe was used for sample injection. HPLC grade Phosphate buffer and Acetonitrile (70 : 30 v/v) were used for the preparing the mobile phase. A freshly prepared (70 : 30 v/v) mixture of buffer and Acetonitrile was used as the mobile phase. The solvents was filtered through a 0.45 µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 mL/min. the column temperature was maintained at room temperature, the detection of the drug was carried out at 217 nm.

Selection of mobile phase

The solution of Rivastigmine was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally phosphate buffer and Acetonitrile (70 : 30 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Rivastigmine.

Preparation of mobile phase

Mobile phase comprised of 20 mM potassium dihydrogen ortho phosphate (Adjusted to pH 3.0 ± 0.05) with Ortho phosphoric acid, and acetonitrile (85 : 15 v/v). Water (pH 7) was used as diluent. Mobile phase was filtered through a 0.45-µm membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1.5 mL/min), which yields a column back pressure of 650-723 psi. Run time was set as 5 min, column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 217 nm and data were acquired, stored and analyzed with the software "Autochro-3000" (Young Lin).

Selection of analytical wavelength

From the standard stock solution, further dilutions were prepared using mobile phase and scanned over the range of 200-400 nm and the spectrum was overlain. It was observed that 217 nm is the λ_{max} for Rivastigmine.

Checking the resolution of drug and material standard

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of Rivastigmine was injected to get the chromatogram. The retention time for Rivastigmine was found to be 3.66 ± 0.25.

Preparation of standard solutions

A stock solution of Rivastigmine was prepared by dissolving Rivastigmine (100 mg) in a volumetric flask (100 mL) containing 25 mL of diluent, sonicated for 20 min and then made up to the volume with diluent. Working standard solution of Rivastigmine (300 µg/mL) was prepared by suitable dilution of stock solution with diluent. Linearity solutions were prepared in diluents containing RS (10-100 µg/mL). Each of these drug solutions (20 µL) was injected 5 times into the column and the peak area and retention times were recorded. The regression of the drug concentrations over the ratios were computed. This regression equation obtained was used to estimate the amount of Rivastigmine in pharmaceutical dosage forms. Solutions containing 10-100 µg/mL of Rivastigmine were subjected to the proposed HPLC analysis to check the inter day and intra day variation of the method by adding known amounts of Rivastigmine to the pre-analyzed samples and then analyzing them by the proposed method.

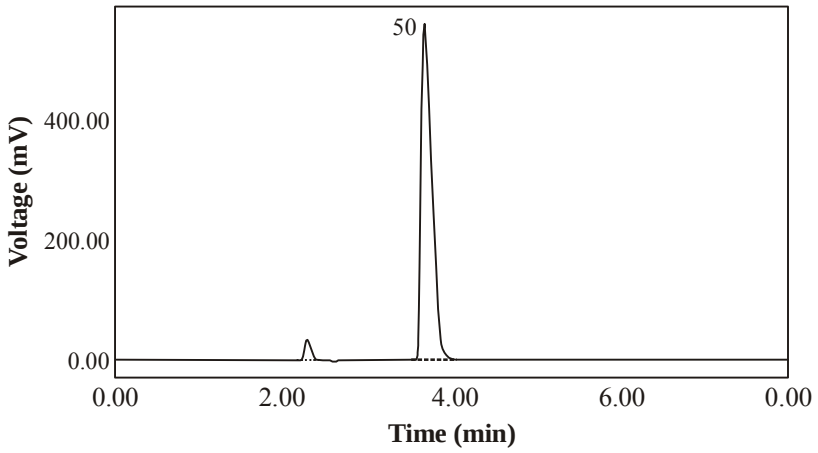
Estimation of rivastigmine in capsules

Two commercial samples of the Capsules containing the drug were chosen for testing the suitability of the proposed method to estimate Rivastigminein Capsules. For this, weigh accurate quantity of the powdered contents of capsule equivalent to about 100 mg of montelukast in to 100 mL volumetric flask, add about 60 mL of diluents, sonicate for about 30 min and dilute to 20 mL with water and methanol. Filter through 0.45 micron filter. The contents of the flasks were made up to the volume with the mobile phase and mixed well. From the above stock, 50 µg/mL sample solution was prepared with mobile phase. Twenty micro liters of each of these solutions was then injected five times in to the column. The mean peak area ratios of the drug to the five such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

RESULTS AND DISCUSSION

The aim of this study was to develop a rapid and precise reverse phase high performance liquid chromatographic method for the estimation of Rivastigmine in its pure form as well as in pharmaceutical dosage forms. Chromatography was carried out on a C_{18}

column (250 x 4.6 mm) using a mixture of Phosphate buffer and Acetonitrile (70 : 30 v/v) (pH 3.0 + 0.05) as mobile phases at a flow rate of 1.0 mL/min the detection was done at 217 nm. The retention time of the drug was 3.6 min. The method produced linear responses in the concentration range of 10-100 µg/mL of Rivastigmine. The method was found to be reproducible for analysis of the drug in tablet dosage forms. The chromatogram is shown in Fig. 1.



Drug	RT (min)	Peak area	Height	Plates	HETP	LOD	LOQ
Rivastigmine	3.66 ± 0.25	3745895	43314	56481	0.0562	0.056 µg/mL	0.166 µg/mL

Fig. 1: A typical chromatogram for Rivastigmine standard solution (50 µg/mL) and its parameters

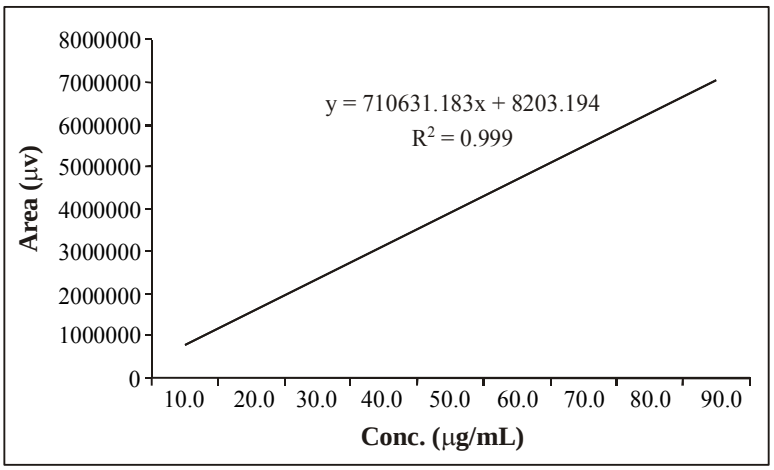


Fig. 2: Variation of area with concentration

Each of the sample was injected 5 times and the same retention time was observed in all the cases. The ratio of the peak areas of Rivastigmine for the different concentrations was calculated and the average value for 5 such determinations are shown in Table 1. The peak area of Rivastigmine was reproducible as indicated by low coefficient of variation. A good linear relationship (r = 0.999) was observed between the concentration of Rivastigmine and the respective ratios of peak areas in the concentration range of 10 to 100 µg/mL of the drug (Fig. 2). The linearity curve was constructed and it's regression coefficient is y = 710631.183 x + 8203.194, when Rivastigmine solutions containing 10 to 100 µg/mL were analyzed by the proposed method for finding out the intra & inter day variations in the recoveries, A low coefficient of variation in the results was observed as shown in Tables 2 and 3. This shows that the present HPLC method is highly precise. The amount of Rivastigmine obtained from the preanalyzed samples containing known amounts of added drug are shown in Table 4. About 100.02% of Rivastigmine could be recovered from the Pre-analyzed samples indicating high accuracy of the proposed method.

Table 1: Calibration of the proposed method

Concentration (µg/mL)	Retention time (min)	Peak area
10	3.667	795623
25	3.598	2145630
50	3.669	3745895
75	3.667	5563248
100	3.662	7123564

Table 2: Intra-day precision for Rivastigmine

Concentration (µg/mL)	Peak area	Mean (n = 5)	S.D	% RSD
50	3756489			
50	3812456			
50	3756231	28226.89	27226.89	0.12
50	3802345			
50	3756123			

Table 3: Inter day precision of Rivastigmine

Concentration (μg/mL)	Peak area	Mean (n = 5)	S.D	% RSD
50	3716489			
50	3712456			
50	3856231	3856231	28226.89	0.15
50	3901345			
50	3756123			

Table 4: Recovery data of Rivastigmine

Amount taken (μg)	Amount found (μg/mL)	Percent recovery	Mean recovery	Percent RSD
20 + 25 = 45	45.01	100.03		
20 + 25 = 45	44.96	99.96	100.01	0.01
20 + 25 = 45	45.06	100.04		
25 + 25 = 50	49.99	99.99		
25 + 25 = 50	50.06	100.04	100.04	0.12
25 + 25 = 50	50.09	100.09		
25 + 30 = 55	55.09	100.06		
25 + 30 = 55	55.03	100.05	100.03	0.25
25 + 30 = 55	54.8	99.99		

The drug content in the capsule was quantified by using the proposed analytical method. The capsule were found to contain an average of 100.03% of the labeled amount of the drug. The low coefficient of variation indicates the reproducibility of the assay of Rivastigmine in dosage forms. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of Rivastigmine in pharmaceutical dosage forms with in a short analysis time. The method was validated by the evaluation of the validated parameters.

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