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METHOD DEVELOPMENT AND PARTIAL VALIDATION OF THE RIVASTIGMINE DRUG IN BULK DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, specific, accurate and precise RP HPLC method has been developed for the determination of Rivastigmine from Bulk dosage form by reverse phase HPLC.C_{18} column (Inertsil, C_{18}, 250 x 4.6mm. 5µ). The sample was analyzed using Potassium phosphate mono basic buffer (pH 2.5± 0.05): Acetonitrile (70:30) as a mobile phase at a flow rate of 1.0ml/min and detection at 217nm. The retention time for Rivastigmine was found to be 3.66 min. The stability assay was performed and was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the 10-100µg/ml of concentration ranges for drug. The method can be used for estimation of Rivastigmine drug in bulk dosage form.

Keywords: Method Development, Validation of Rivastigmine, Bulk Dosage Form**.**

INTRODUCTION

Rivastigmine is a 3-[(1S)-1-(dimethyl amino) ethyl]phenyl N-ethyl-N methyl 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 holinesterase (eg. Acetylcholinestrase, butyrylcholinesterase), preventing the hydrolysis of acetylcholine, and thus leading to an increased concentration of acetylcholine at cholinergic synapses. The anticholinesterase activity of Rivastigmine is relatively specific for brain acetylcholinestrase and butyrylcholinesterase compared with those in peripheral tissues. Literature survey reveals that there is no method available in Hplc methods for estimation of Rivastigmine. Method has been reported for the estimation of Rivastigmine in Bulk dosage form. Present work emphasizes on the stability testing of Rivastigmine in Bulk dosage form by RP-HPLC (Anonymous 1 and 2; David G. Watson, 1999).

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EXPERIMENTAL

A High performance liquid chromatography system, the purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecylsilane chemically bonded to porous silica particles of 5µm diameter reverse phase C18 column (Inertsil, C_{18}, 250 x 4.6mm. 5µ.). Optimized chromatographic conditions are listed in Table 1.

MATERIALS AND CHEMICALS

Pure samples of USP Rivastigmine RS were obtained from Merkfor the estimation Rivastigmine in commercial formulations. HPLC grade Monobasic Potassium phosphate, Hexane sulphonic acid sodium salt, Phosphoric acid solution and Acetonitrile were procured from institute and of RankemLtd. High pure water prepared by using Millipore Milli Q plus purification system.

Preparation of Standard Stock Solution

A stock solution ofRivastigmine 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 (300µg/ml) was prepared by suitable dilution of stock solution with diluent. Linearity solutions were prepared in diluents containing RS (10-100µg/ml). Each of these drug solutions (20µl) was injected into the column and the peak area and retention times were recorded.

Linearity

Aliquots ranging from 10-100µg/ml were prepared by suitable dilution of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for Rivastigmine, the higher concentration range was used to improve signal to noise ratio. Linearity was determined by analyzing five working standard solutions over the concentration range of 10-100µg/ml for Rivastigmine.

Preparation of Sample Solution

Transfer an accurately equivalent to 100mg of Rivastigmine, to a100ml volumetric flask, dilute it about 70 ml of diluents and mix well. Then mixed up the volume with diluents.Final concentration about 300µg/mL. (Rivastigmine Sample concentration of about 300µg/mL).

Validation of the Method

The method was validated in terms of system precision, linearity, precision and specificity of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Rivastigmine and measured the absorbance at 217nm. Calibration curves where constructed by plotting the area against the concentration. Rivastigmine shows the linearity in the concentration range from 10-100µg/ml of concentration with correlation coefficient of 0.999.Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions (Beckett AH and Stenlake JB, 1999; David Harvey, 2000; James W Munson, 2001; Hobert H Willard, 1986).

RESULTS AND DISCUSSION

To evaluate the linearity range of Rivastigmine, varying concentrations of standard solution s is diluted ranging from 10-100µg/ml of the concentration were injected into HPLC system. The linearity graph was plotted. A calibration curve was constructed for each sample by plotting the peak area obtained the concentration. The linearity data for Rivastigmine are presented as follows table-2 and fig-1.

System Suitability

To determine the suitability of chromatographic system described for the method of analysis by establishing system suitability parameters like peak tailing factor, number of theoretical plates and %RSD of Rivastigmine Standard solution on daily.

Precision

Five sets of aliquots with same concentration (50 µg/ml) were prepared and these solutions were analyzed to record any intra and inter day variations in the results. The results obtained for Intra and interday variations are shown in Tables 4 and 5 respectively.

Specificity

To establish the identification peaks, which are not interfere with main peak.

Procedure

Inject separately 20µl of blank, standard, samples were prepared (Douglas A. Skoog, 1979; Frank A Settle, 2004) under into the chromatograph and measure the responses for all peaks for all solutions. The interference between peaks obtained for all the solutions should be NIL. The peaks should well separate from the peaks obtained with blank, standard and sample solution.

Solution Stability

The Rivstigmine sample and standard solutions were prepared. Replicate injections of the standard solution were made at the following time intervals: initial, 24 hours. The values were compared (Fengshan Yu *et al*., 2009; Gary D. Christian, 2004; ICH Harmonized Tripartite Guidelines) to initial standard area and they are tabulated. Sample solution were injected at 24 hours time interval, and %area difference was compared (Frank Settle, 2004; Kaur H, 2006) to the initial area generated by these samples and are tabulated (Table 6 & 7).

Table 1. Optimized Chromatographic conditions

Parameter	Optimized condition
Instrument	Quaternary isocratic HPLC (Younglin HPLC YL9000 series) with YL 9110 Pump and with "autochro 3000" software and UV-Vis detector YL9120.
Column	Inertsil, C-18, 250 x 4.6mm. 5µ.
Mobile phase*	Potassium phosphate mono basic buffer (pH 2.5± 0.05): Acetonitrile (70:30)
Flow rate	1.0ml/min
Detection	217nm
Injection volume	20µl
Temperature	Ambient
Run Time	5 minutes

*Filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated

Table 2. Linearity of Rivastigmine

S.No	Concentration(µg/ml)	Retention Time(min)	Peak Area
1	10	3.667	795623
2	25	3.598	2145630
3	50	3.669	3745895
4	75	3.667	5563248
5	100	3.662	7123564

Table 3. System Suitability Parameters

S No	Drug	RT (min)	Peak Area	Height	Plates	HETP
1	**Rivastigmine**	3.66	3745895	43314	56481	0.0562

Table 4. Intra-day Precision for Rivastigmine

Concentration(µg/ml)	Peak Area	Mean(n=5)	S.D	% RSD
50	3756489	3776729	28226.9	0.74739
50	3812456			
50	3756231			
50	3802345			
50	3756123			

Table 5. Inter day precision of Rivastigmine

Concentration(µg/ml)	Peak Area	Mean(n=5)	S.D	% RSD
50	3716489	3856231	28226.89	0.73
50	3712456			
50	3856231			
50	3901345			
50	3756123			

Fig. 1. Linearity Curve of Rivastigmine

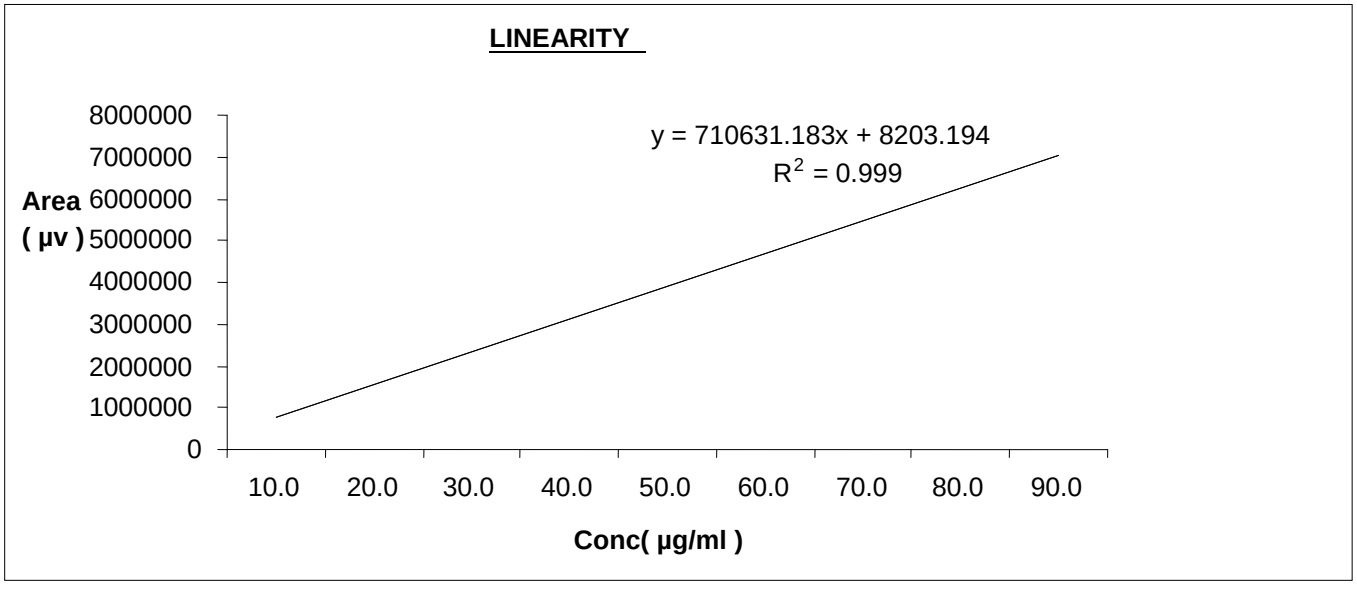
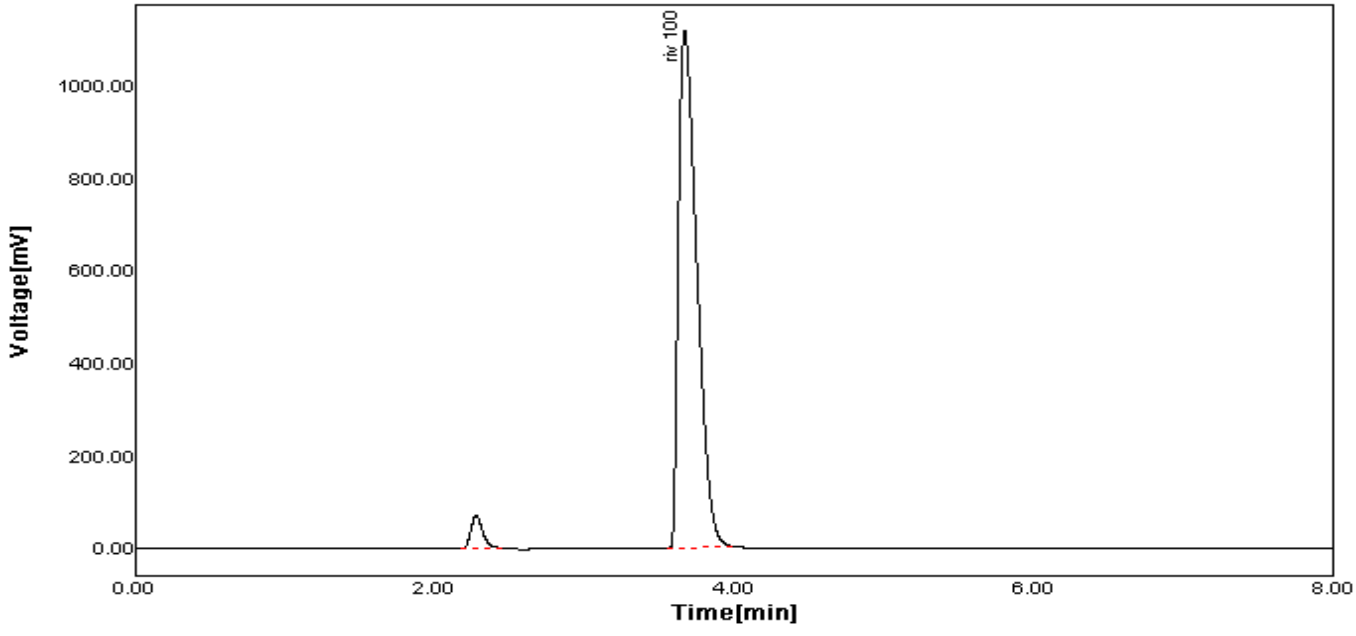


Fig.2 A typical chromatogram for Rivastigmine



CONCLUSION

The proposed HPLC method validation for estimation of Rivastigmine in bulk dosage form is carried out as per ICH and USP Guidelines. System suitability test id established and recorded, the method found to be specific for validation of estimation of Rivastigmine in bulk dosage form. The method found to be linear in the specified range. Hence, this method stands validated and can be used for routine analysis.

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