Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(11):436-442

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Analysis of rivastigmine in *in vitro* transdermal permeation studies by RP-HPLC-PDA method

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ABSTRACT

The aim of the present work is to develop and validate a simple, efficient, economical RP-HPLC method for the analysis of Rivastigmine (RVS) in drug in adhesive type transdermal patches and in transdermal permeation and/or release studies. An Inertsil-ODS-3 column (250 x 4.6mm, 5µm) with mobile phase containing 10mM ammonium acetate: methanol (35:65% v/v) at a flow rate of 1.0mL/min was used in isocratic mode and eluents were monitored at 217nm. The retention time of RVS was 5.32 min and showed a good linearity in the concentration range of 2-10µg/mL with a correlation coefficient >0.999. The validation parameters like specificity, linearity, accuracy and limit of detection, limit of quantification, precision, robustness fulfilled regulatory requirements. The percent recoveries were ranged in between 98-102, (RSD < 2). The developed HPLC method was successfully used for the analysis of RVS in bulk and in house made transdermal patches and in transdermal permeation and/or release studies.

Keywords: Inertsil-ODS-3 column, Method validation, PDA detection, Rivastigmine, Transdermal permeation studies.

INTRODUCTION

RVS is a cholinesterase inhibitor and chemically it is (s)-3-[1-(dimethyl amino) ethyl] phenyl N-ethyl-N-methyl carbamate and used in the treatment of mild to moderate dementia of the alzheimer's type and dementia due to parkinson's disease [1]. It acts by inhibiting the acetyl cholinesterase enzyme from breaking down acetylcholine, and it is a CNS selective [2]. Various analytical methods have been reported in the literature for quantitative determination of RVS by HPLC [3-6], stability indicating HPLC [7], spectrophotometry [8], spectroflourimetry [9], LC-MS [10-12] and by GC-MS [13]. Literature survey reveals that there were no validated RP-HPLC/PDA methods reported for the estimation of RVS in dosage forms like transdermal patches and in transdermal permeation and/or release studies. Hence, the present investigation was aimed at developing a validated RP-HPLC-PDA method for the analysis of RVS in transdermal patches and in transdermal permeation and/or release studies.

EXPERIMENTAL SECTION

Chemicals

RVS was gift sample from Sodhana laboratories, Hyderabad, India. Ammonium acetate, water and methanol were purchased from E. Merck, Mumbai, India. Sodium chloride was purchased from SDFCL, Mumbai, India. All the solvents and reagents were of HPLC grade.

Equipment
A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Inertsil-ODS-3 column (250 × 4.6mm, 5µ).

Transdermal Permeation/Release Studies
The *in vitro* transdermal permeation studies were performed using a vertical type Transdermal Diffusion Cell apparatus with a water circulation system, a water heater and an eight stage magnetic stirrer (Orchid Scientifics, Nashik, India). Franz diffusion cells with a diffusion area of 1.77 cm^2 and a receptor volume of ~14 mL were used and 0.9%w/v sodium chloride (saline) was used as receptor fluid. Circular drug in adhesive type RVS patches of 2.19 cm^2 were cut and release liner was removed and placed between receptor and donor compartments facing backing membrane side to the donor compartment. The surface of the patch was maintained at 32°C by keeping receptor fluid at 37°C using a circulation water bath. Samples of 500 µL were withdrawn at pre-determined time intervals and stored at 4°C until analysis. 500 µL of fresh saline was replaced at each sampling point. The study was carried out over a 24 hr period and the samples were analyzed for RVS content by developed HPLC method.

Chromatographic Conditions
Mobile phase consisting of 10mM ammonium acetate: methanol (35:65% v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min. before use. The flow rate was 1 mL/min and the injection volume was 10µL. PDA detection was performed at 217nm and the separation was achieved at ambient temperature.

Preparation of stock and standard solutions
The stock solution of RVS strength 1mg/mL was prepared by dissolving 10 mg of drug in methanol and volume was adjusted to the mark with the same. An appropriate volume of the stock solution was then further diluted with 0.9%w/v sodium chloride to get the required concentrations of standard solutions at a concentration range of 2-10µg/mL.

Validation of the HPLC method
The proposed method was validated as per ICH guidelines.

Linearity
A linear relationship was evaluated across the range of the analytical procedure with a minimum of five concentrations. A series of standard dilutions of RVS were prepared over a concentration range of 2-10µg/mL (2, 4, 6, 8, 10µg/mL) from stock solution and injected in triplicate. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the test results were evaluated by appropriate statistical methods where by slope, intercept, and regression (R^2) correlation coefficients (R) were calculated and the data was given in Table-1.

Precision
Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration. The standard deviation and the relative standard deviation were reported for precision. Less than 2% RSD for peak areas indicates the precision of the developed method and the data was presented in Table-1.

Specificity
The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that obtained from the samples obtained from *in vitro* transdermal permeation samples using PDA detector. The overlay of diluent, standard and sample were presented in Figure-2. Absence of interference of excipients in the transdermal patch indicates the specificity of the proposed method.

Accuracy
Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed method recovery studies were performed by the standard addition method by spiking 80%, 100%, 120% of the known quantities of standard within the range of linearity to the synthetic solution of drug product (6µg/mL) and these solutions were analyzed by developed method in triplicate. The % recovery and the %RSD were calculated at each level of addition and the data was given in Table-1.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
LOD and LOQ were calculated based on calibration curves. They were expressed as LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (Where, σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

Robustness
To determine the robustness of the method developed, the experimental conditions were deliberately altered and the chromatographic parameters viz., tailing factor, no. of theoretical plates were recorded. The flow rate of the mobile phase was 1mL/min. To study the effect of flow rate, the flow rate was changed by 20%, the effect of wavelength was studied by changing wavelength by ±2nm, the effect of mobile phase was studied by changing the mobile phase by 20% and the data was given in Table-2.

System suitability
System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50µL. The system suitability test parameters were noted and % RSD was calculated.

Assay
Samples from 4 different batches of transdermal patches were transferred into a 10mL volumetric flask with methanol and vortex for 5min and volume was adjusted up to the mark with methanol. The above solution was centrifuged and then filtered using nylon disposable syringe filter (13 mm, 0.45µm). An aliquot of filtrate was diluted with 0.9% sodium chloride and analyzed in triplicate. The amount present in the each batch of transdermal patch was quantified by comparing the area of standard RVS with that of the sample.

RESULTS AND DISCUSSION

Various HPLC-UV and LC-MS methods were published for the estimation of RVS in bulk and dosage forms However; no methods were reported on the quantification of RVS in transdermal dosage forms. Hence, the present investigation was aimed to develop a simple, economical RP-HPLC-PDA method for the determination of RVS in bulk and transdermal patches and in transdermal permeation and/or release studies.

Method Development
Initially mobile phase optimization was carried out with Phenomenex C_{18} column (150 x 4.6 mm) using 10Mm Ammonium acetate and methanol (30:70v/v) as mobile phase at a flow rate of 1 mL/min and ammonium acetate was used as diluent and the eluents were monitored at 217nm. With these conditions a broad peak of RVS was eluted at 2.68 min. In another trial Apollo C_{18} column (150 x 4.6 mm) was used with 5mM ammonium acetate and methanol (25:75v/v) as mobile phase at a flow rate of 1mL/min and with these conditions RVS was eluted at 4.09 min but tailing was observed. Finally, the mobile phase of 10mM ammonium acetate and methanol (35:65% v/v) with Inertsil ODS column (250 x 4.6mm, 5µ) was selected at flow rate of 1mL/min using 0.9% NaCl as diluent and under these conditions sharp RVS peak was eluted at 5.32 min with good symmetry and tailing factor was within the limits. For quantitative purpose wavelength was set at 217 nm, which provided better reproducibility without interference. The peak purity index was found to be greater than 0.9999 and this indicating peak purity of the drug sample used in the analysis and the RVS chromatogram along with UV spectrum and peak purity profile was shown in Figure-1.

Method validation
The method has been validated as per ICH-Guidelines for following parameters.

Linearity
The range of reliable quantification was set at the concentrations of 2-10µg/mL of RVS. This range was selected based on 80-120% of the standard concentration used for accuracy and were analyzed in triplicate. Peak areas and concentrations were subjected to least square regression analysis to calculate regression equation. The correlation coefficient (R) was found to be 0.999 indicating a linear response over the range used. The data from the calibration curve was given in Table-1.

Precision
Precision studies were carried out in terms of repeatability. Repeatability of standard application was assessed by using six replicates of concentration at 6µg/mL level and the data was given in Table-1. The % RSD was found to be below 2 for peak areas, this shows the closeness of the data values to each other, indicating the precision of the method.

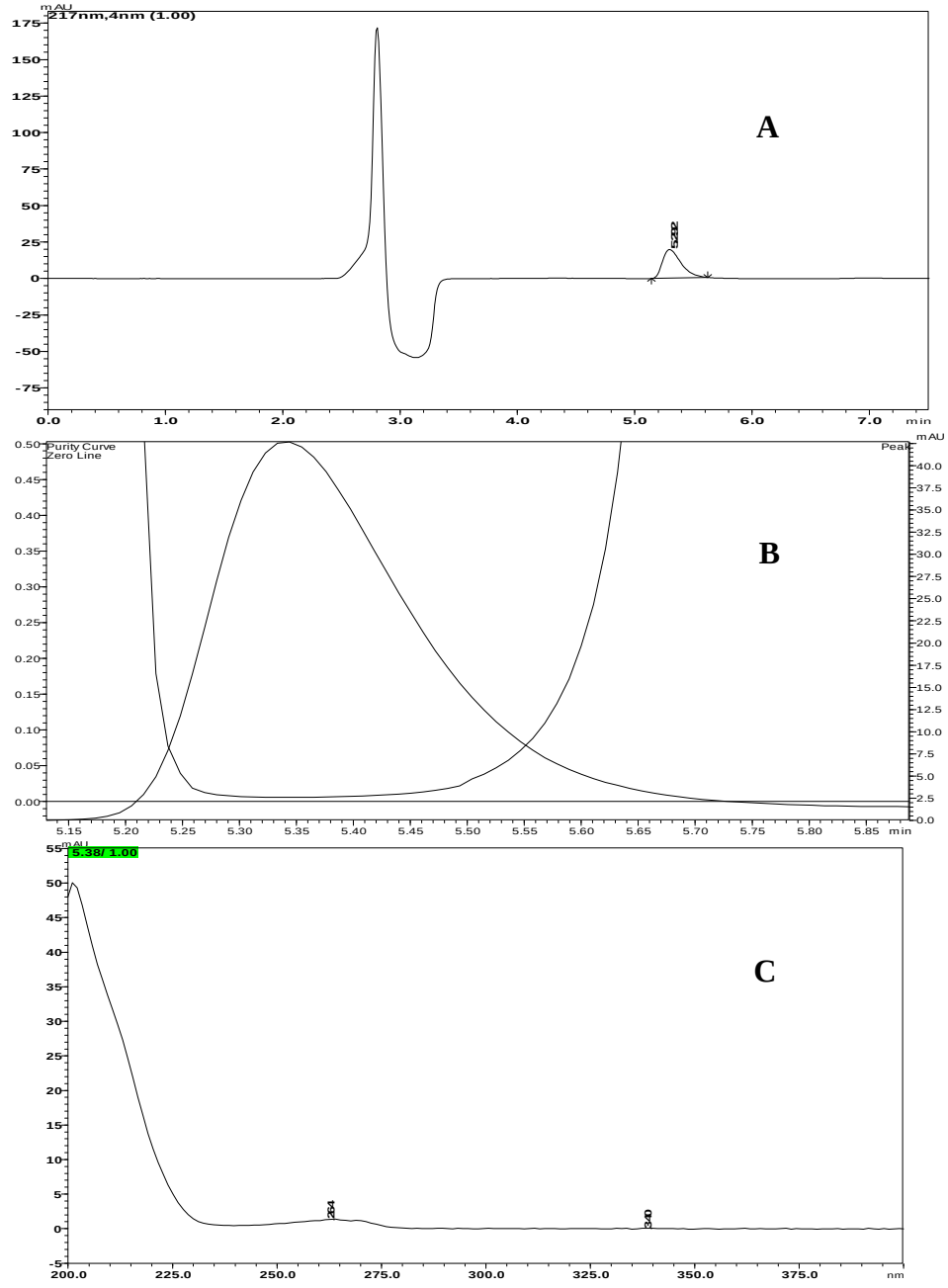

Fig. 1: A - Chromatogram of RVS (6µg/mL); B - Peak purity index of RVS and C - UV spectrum of RVS

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies by standard addition method by spiking the known quantities of standard at 80%, 100%, 120% to the drug product solution of 6µg/mL and these solutions were analyzed in triplicate in each level of addition. The %RSD and the %Recovery were within the acceptable limit in all cases. It is evident from the results of accuracy study given in Table-1, that the proposed method enables very accurate quantitative estimation of RVS.

Specificity

The specificity of the method was established by injecting the solutions of diluent, standard, sample (transdermal patch) individually to examine any interference, from the overlay of chromatograms as shown in (Figure-2) it can be inferred that there were no co-eluting peaks at the retention time of RVS. These results indicate that the peak of analyte was pure and the *in vitro* transdermal permeation samples did not show any interference with the sample analysis. The peak purity indices for sample and standard were found to be greater than 0.999 and this confirms specificity of the method by PDA detector.

Table-1: Linearity, Precision and Accuracy data of RVS

	Parameters	RVS
Linearity(n=3)	**Range**	2-10µg/mL
	Régression équation	y=43228x+43711
	Régression coefficient (R^2)	0.999
	Corrélation coefficient (R)	0.999
Accuracy(n=3)	**%Level of Addition**	**Mean Recovery (%RSD)**
	80	98.61 (0.45)
	100	100.59 (0.34)
	120	101.19 (0.12)
Precision(n=6)		
System Precision	**Average Peak area of the standard sample(%RSD)**	215227 (1.18)
Method Precision	**Average peak area of the Assay sample(%RSD)**	215305.7(0.39)

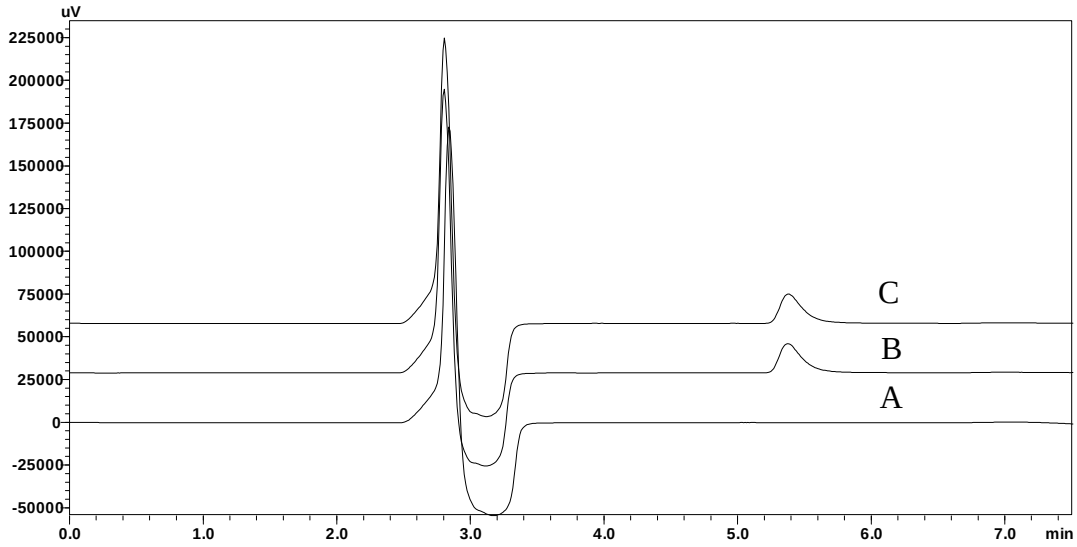

Fig. 2: Overlay of the Diluent (A), RVS Standard (B) and RVS Sample (C) chromatograms

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on statistical calculation from the calibration curves, where LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves). The limit of detection and limit of quantification were found to be 0.1911µg/mL and 0.5792µg/mL respectively and the RVS peaks were detected without any base line disturbances at this concentration.

Table-2: Robustness data of RVS

Chromatographic parameter	Retention time (min)	Theoretical plates #	Tailing factor (T_f)
Flow rate (mL/min)			
0.8	6.450	5716.908	1.524
1.0	5.321	4891.004	1.499
1.2	4.459	4385.572	1.468
Wavelength (nm)			
215	5.321	5716.908	1.482
217	5.321	4891.004	1.483
219	5.321	4385.572	1.483
Mobile phase			
63:37	5.523	5161.327	1.526
65:35	5.272	5107.446	1.482
67:33	5.087	5048.946	1.499

Robustness

As part of the robustness, a deliberate change in the flow rate, wavelength and mobile phase was made to evaluate the impact on the method. Retention time was significantly changed with flow rate, mobile phase and no change in the retention time was observed in wavelength change.

The parameters like theoretical plate number, tailing factor were not changed and were within the limits. These results indicated that the method is robust in terms of changed flow rate, mobile phase and wavelength.

System suitability

System suitability testing is an integral part of the analytical procedure. System suitability studies were carried out by injecting five times a 6µg/mL standard concentration of RVS at different injection volumes ranging from 10µL to 50µL. The %RSD values for system suitability test parameters like retention time [R_t = 5.344 (0.3264%)], tailing factor [T_f = 1.216 (0.9139%)] and theoretical plate number [# = 4619.315 (0.04050%)] were less than 2 indicating the present conditions were suitable for the analysis of RVS in transdermal patch.

Assay

Assay of in house made RVS transdermal patch was performed by the proposed method and the % assay of in house made RVS transdermal patch was calculated as an average of 3 determinations, which was about 100.44 ± 0.370. These results indicate that the present HPLC method can be successfully used for the assay of RVS in bulk and transdermal patch.

Stability of the stock solution

The stability of the stock solution was determined by analyzing the samples under refrigeration (8±1^{o}C) at different time intervals up to 48hrs. The variation in assay values at different time intervals were found to be less than 2% of the initial zero time interval solution, thus indicating that the solutions were stable for a period of 48hrs when stored at 8^{o}C.

In vitro transdermal permeation/release studies

The validated method was used for the analysis of RVS in samples obtained from *in vitro* transdermal permeation/release studies. No interference peaks were observed with the RVS peak. The cumulative percent drug release profile of RVS from a drug in adhesive type transdermal patch prepared using DUROTAK 87-2510 adhesive was presented in Figure 3. The method was successfully used for the quantification of RVS in the *in vitro* transdermal permeation/release studies.

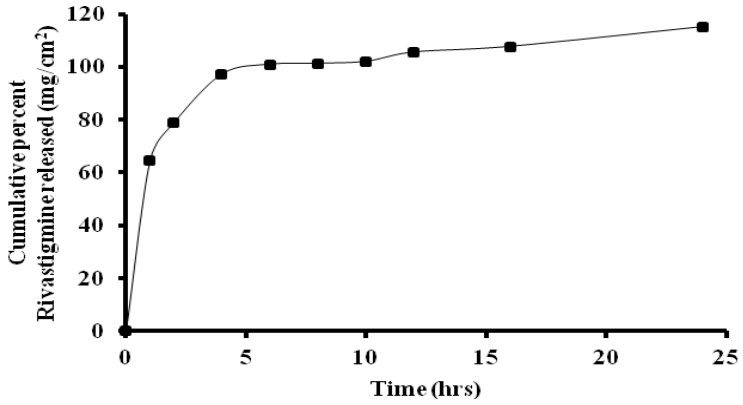

Fig.3: *In vitro* drug release profile of RVS

CONCLUSION

In this work, a simple and efficient RP-HPLC-PDA method was developed for the analysis of RVS in bulk and transdermal patch and in transdermal permeation and/or release studies. The method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of RVS in transdermal patch using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity, proved to be with in the limits. The method provides selective quantification of RVS without interference from diluent. Therefore, this method can be employed in quality control to estimate the amount of RVS in bulk and transdermal patch and in transdermal permeation and/or release studies.

Acknowledgements

The authors are thankful to Sodhana Laboratories, India for providing gift sample of RVS and also to the Siddhartha Academy of General and Technical Education, Vijayawada, for providing necessary facilities to carry out this research work**.**

REFERENCES

[1] Maryadele JO Neil. The Merck Index. 13th ed. Merck Research Lab publishers, White House Station, NJ, US, **2001,** 1480 [8323].
[2] HP Rang; MM Dale; JM Ritter; RJ Flower; G Henderson. Pharmacology. 7th ed. Elsevier, Toronto. **2012**, 483, 484.
[3] A Karthik; GS Subramanian; M Surulivelrajan; AR Kumar; SB kamat. *Arzneimittel Forschung*.**2008,** 58(5), 205-10.
[4] K Arumugam; MR Chamallamudi; RR Gilibili; R Mullangi; S Ganesan; SS Kar; et al. *Biomedical Chromatography*.**2011**, 25(3), 353-361.
[5] S Navaneethakrishnan; MD Reshma Begum; PS Kumar; JB David; MP Kumar. *Der Pharmacia Sinica***.2012,** 3(2), 295-299**.**
[6] K Arumugam; MR Chamallamudi; SR Mallayasamy; R Mullangi; S Ganesan ;L Jamadar; A Ranjithkumar; N Udupa. *Journal of young pharmacist*.**2011** 3(4), 315-321.
[7] AS Patil; PB Patil; DB Shinde; BR Chaudhari. *International Journal of Pharmacy and Biomedical Sciences.***2012,** 3(3), 122**.**
[8] MY Salem; AM Kosasy; MG Bardicy; MKA Rahman. *Drug Test Analysis.***2010** 2(5), 225-33.
[9] R Kapil; S Dhawan; Bhupinder Singh. *Indian Journal of Pharmaceutical Sciences*.**2009,** 71(5), 585-589.
[10] J Bhatt; G Subbhaiah; S Kambli; B Shah; S Nigam; M Patel; A Saxena; A Baliga; H Parekh; G Yadav. *Journal of Chromatography.B, Analytical Technologies in the Biomedical and Life Sciences.***2007,** 852(1-2), 115-21.
[11] A Enz; A Chappius; A Dattler. *Biomedical Chromatography*.**2004**, 18(3), 160-6.
[12] F Pommier; R Frigola*. Journal of Chromatography B.***2003**, 784(2), 301-313.
[13] Y Sha; C Deng; Z Liu; T Huang, B Yang; G Duan. *Journal of Chromatography.B, Analytical Technologies in the Biomedical and Life Sciences.***2004**, 806(2), 271-6.