INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.4.2022.12(43)

A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF GALANTAMINE HYDROBROMIDE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new RP-HPLC method for the estimation of Galantamine hydrobromide in pharmaceutical formulations is developed and validated. The separation is achieved through *Inertsil* ODS-3V column (150 mm × 4.6 mm, 5µ) using a solvent mixture of phosphate buffer and acetonitrile in a ratio 75:25 v/v at a flow rate of 1.0 ml/min. The drug is detected at 230 nm and the retention time is observed at 4.2 min. The method is validated according to ICH Q2 (R1) guideline. The method is linear in the concentration range 6-30 µg/ml with excellent correlation coefficient (r^2 =0.999). Accuracy results are found from the recovery studies and the mean percentage recovery values are in between 99.43% to 99.2%. The results of intra-day and inter-day precision studies shows that % RSD is not more than 2 %, which indicates that the proposed method has good reproducibility. The proposed method can be used in the quality control lab for the estimation of Galantamine hydrobromide in bulk and pharmaceutical dosage forms.

Keywords: Galantamine, Validation, Chromatography, Pharmaceutical and Accuracy.

INTRODUCTION

Galantamine hydrobromide is a parasympathomimetic, and it acts as reversible, competitive acetylcholinesterase inhibitor. Chemically it is (4aS,6R,8aS)-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol hydrobromide (figure 1). It is used in the treatment of Alzheimer's disease. A thorough literature survey revealed that there are a few analytical methods developed for the estimation Galantamine hydrobromide by [UV][1] and [HPLC][2-5] in pharmaceutical formulations. The aim of the present research work is to develop and validate the assay method for the estimation of Galantamine hydrobromide in pharmaceutical formulations.

MATERIALS AND METHODS

Instruments

A *Shimadzu* HPLC LC 100 series equipped with UV detector, *Inertsil* ODS-3V, (150 mm × 4.6mm, 5µ) column and *Rheodyne* injector is used. A *Systronics* digital pH meter is used for pH adjustments. A *Shimadzu* double beam spectrophotometer is used for spectral measurements. Nylon membrane filters with mean pore size 0.22 µ is used for solvent

pretreatment.

Reagents

All the chemicals and reagents (disodium hydrogen orthophosphate, orthophosphoric acid) used are analytical grade and the solvents used in this study are HPLC grade methanol, acetonitrile and water.

Preparation of the solutions

Preparation of buffer solution

A 5.34 g of disodium hydrogen orthophosphate dehydrate is accurately weighed and dissolved in 500 ml of water. Then it is transferred into a 1000 mL volumetric flask, and the final volume is adjusted with water. The pH of the solution is adjusted with dilute orthophosphoric acid and then filtered through 0.45 µm membrane filter. Solution A is prepared by a mixture of buffer and methanol in the volume ratio of 95: 5(v/v) as solution and degassed through filtration.

Solution B is prepared by a mixture of acetonitrile and methanol in the volume ratio of 95: 5(v/v) as solution and degassed through filtration.

Preparation of mobile phase

Mobile phase is prepared by mixture of solution A and solution B in the volume ratio of 75: 25 (v/v) and degassed through filtration.

Preparation of diluent

Diluent is prepared by mixture of methanol and water in the volume ratio of 95: 5 (v/v) and degassed through filtration.

Preparation of standard solution

About 24.0 mg of Galantamine hydrobromide is accurately weighed and transferred into a 100 mL volumetric flask. To this 30 mL of diluent is added and sonicated. The solution is allowed to equilibrate to room temperature and then diluted to final volume with diluent. Working standard solutions are prepared by successive dilution.

Preparation of sample solution

A capsule content equivalent to 24.0 mg of Galantamine hydrobromide is accurately weighed from twenty randomly selected capsules. The weighed contents are carefully transferred into a 100 mL volumetric flask. 30 ml of the diluent is transferred and the contents are dissolved by sonication with occasional shaking for 20 minutes. The final volume is adjusted with diluent. The solution is filtered through a 0.45 µm Nylon filter. The first three ml is carefully discarded then 10 ml portion is transferred into a 100 ml flask and the final volume is adjusted with diluent. The sample solution thus obtained is degassed through sonication for 10 minutes. Working sample solutions are prepared by successive dilution.

Evaluation of System Suitability

The HPLC system is stabilized for thirty min. by following the chromatographic conditions to get a stable base line. One blank followed by six replicates of a single standard solution is injected to check the system suitability. The column efficiency as determined from Galantamine hydrobromide peak is not less than 2000 plate count and the tailing factor for Galantamine hydrobromide peak is not more than 2.0. The relative standard deviation for the peak areas of the six replicate injections is not more than 2.0 %.

Preparation of standard curve

Separately a 20µl of the Blank and five standard solutions of 6.1, 12.3, 19.6, 24.5and 29.4µg/ml of are injected into the liquid chromatograph and the chromatograms are obtained. The retention time and average peak areas are recorded. Calibration graph is plotted by taking concentration of Galantamine hydrobromide on X-axis and peak areas on Y-axis.

Estimation of drug in pharmaceutical formulations

The content of twenty capsules is accurately weighed. From this capsule powder an amount equivalent to 10 mg of Galantamine hydrobromide is taken and the drug is extracted in 10 ml of mobile phase by sonication for a period of 20 minutes. This solution is filtered through 0.45 µm nylon membrane filter. The filtered solution is suitably diluted for analysis and injected into the liquid chromatograph and the chromatograph is recorded. Calculation of the amount of each drug is done by using the calibration curve.

RESULTS AND DISCUSSION

Method development and optimization

The extent of the organic and aqueous phase is adjusted to attain a specific and reliable assay method for the determination of Galantamine hydrobromide with less run time, short retention time and the sharp peak. The mobile phase consisting of phosphate buffer and acetonitrile in a ratio 75:25 v/v in isocratic mode offered a good separation at ambient temperature. Under these conditions, using a flow rate of 1.0 mL/min and a runtime of 8 min the proposed method is simple and do not

involve laborious time-consuming sample preparation. The UV absorption of the Galantamine hydrobromide shows good response at 230 nm (Fig. 2). The optimized chromatographic conditions is given in table 1. The method is validated as per ICH Q2 (R1) guidelines.

System suitability studies

It is observed from the data in table 1 that system suitability parameters, tailing factor is less than 2.0 and theoretical plates are more than 2000. Both parameters are found to be within the acceptance criteria. Hence, it is evident that the preferred system is suitable.

Specificity

No interference from placebo is observed at the retention time of Galantamine hydrobromide. Therefore it can be evident that no interference due to placebo and standard for the quantification of Galantamine hydrobromide in formulations. Hence, the method is specific and selective. The results are shown in Fig. 3.

Linearity

The proposed method is linear over the concentration range 6-30 µg/mL and the correlation coefficient between the concentration of selected drugs and their chromatographic peak response (area) is highly impressive and found to have R^2 = 0.999. This regression analysis indicates that the method has excellent linearity over the wide concentration range. The linearity plot is given in fig.4. The results of the linearity is shown in table 2.

Limit of detection and limit of quantification

The limit of detection and quantitation is determined from the linearity plot and it is found satisfactory since the lowest amount that can be detected by this method is 0.135 µg/mL and the minimum concentration of the analyte that can be quantified is found as 0.411 µg/mL.

Precision

Three different sample concentrations and triplicate of each concentration in linearity range are taken for intra and inter day precision studies. The % RSD for intra-day precision of the sample (n=6) is found as 0.2. The interday precision is achieved by performing the method in between the days and the % RSD is found as 0.18. In all the cases studied, the % RSD has been found by the proposed method is within 2.0 % that it indicates a consistency in its precision and can be seen in the results of precision studies in table 5.

Accuracy

Recovery studies are performed on three different levels at 25, 50, 80, 100, and 120 in three replicates in each level in the present study. Standard drug is spiked to the pre-analyzed sample and injected into an HPLC system to determine the amount recovered by the proposed method. The % recovery values are observed to be in the range of 99.74 % - 99.89 % and the mean % recovery of the drug is found to be 99.83. The % RSD is observed to be less than 1.0 at every spiking level. Therefore, the recovery results indicated that the method has an acceptable level of accuracy. Results for the accuracy study are shown in table 4 and the chromatograms are given in figure 5.

Robustness

Robustness of the method is determined by small deliberate changes in flow rate, mobile phase ratio and column oven temperature. The content of the drug is not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method is robust. The results of robustness are presented in Table 5.

CONCLUSION

The present method enables the estimation of Galantamine hydrobromide in pharmaceutical formulations and the proposed RP- HPLC conditions ensure excellent linearity, accuracy and the precise quantification of the drug. Results from statistical analysis are indicative of satisfactory in reproducibility. Hence, this RP-HPLC method can be used for quality control laboratory for quantitative analysis of Galantamine hydrobromide.

ACKNOWLEDGEMENTS

The authors are thankful to Spectrum Labs, Hyderabad, India for providing reference standards and all facilities to complete this research work. The authors are also thankful to QIS College of Pharmacy, Ongole, and Andhra Pradesh, India for providing all facilities and encourage us to do this research work.

Table 1: Optimized chromatographic conditions

and system suitability

Parameter	Conditions
Stationary phase	*Inertsil* ODS-3V (250 x 4.6 mm, 5µm)
Mobile phase	Solution A:0.05M disodium hydrogen phosphate dihydrate : Methanol(95:5v/v) Solution B: Acetonitrile: Methanol (95:5v/v). Solution A :Solution B (75:25v/v)
Flow rate (mL/min)	1.0
Column back pressure (kg/cm^2)	80 – 85
Run time (min)	8
Column temperature (^{0}C)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	λ*max* at 230 nm, UV
Efficiency (N)	3285
Tailing factor (As)	1.15

Table 2: Linearity data of galantamine hydrobromide

Actual concentration (%)	Concentration (µg/mL)	Mean area	S.D N=3	%RSD N=3
25	6.1	191535	414.70	0.2
50	12.3	381677	387.59	0.1
80	19.6	621311	751.96	0.1
100	24.5	760240	600.51	0.1
120	29.4	914341	772.69	0.1

Table 3: Intra-day precision studies

Sample	Area	Sample weight	% Assay
1	1003919	302.12	101.05
2	1002431	301.72	100.4
3	1002507	301.92	100.7
4	1003782	302.12	100.8
5	1003857	302.12	100.9
6	1004124	303.12	101.7
Mean			100.99
SD			0.37
% RSD			0.36

Table 4: Accuracy results of galantamine hydrobromide

Spiking level (%)	Spiked concentration (µg/mL)	Area	Recovered concentration (µg/mL)	Mean % recovery
25	6.80	211771	6.79	99.89
	6.80	212056	6.78	
	6.80	211057	6.81	
50	12.1	377700	12.01	99.74
	12.1	378056	12.12	
	12.1	376133	12.09	
100	24.30	756311	24.21	99.87
	24.30	756468	24.19	
	24.30	757270	24.31	
Mean				99.83
SD				0.081
% RSD N=3				0.082

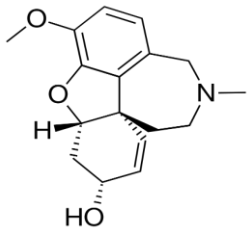

Fig. 1: Structure of Galantamine Hydrobromide

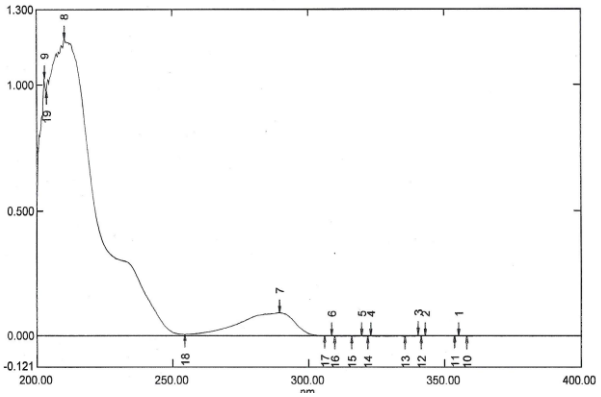

Fig. 2: λ_{max} curve for Galantamine Hydrobromide

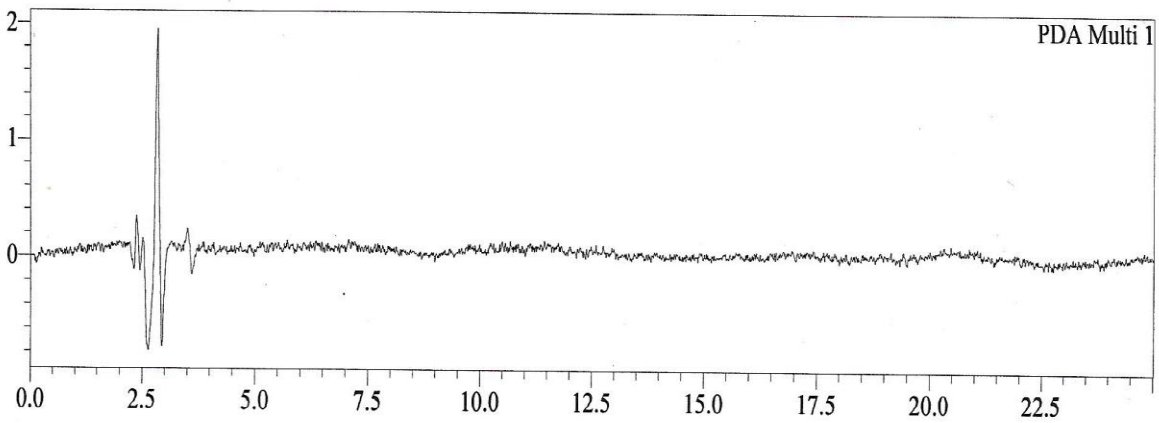

Fig. 3: Placebo Chromatogram of Galantamine Hydrobromide

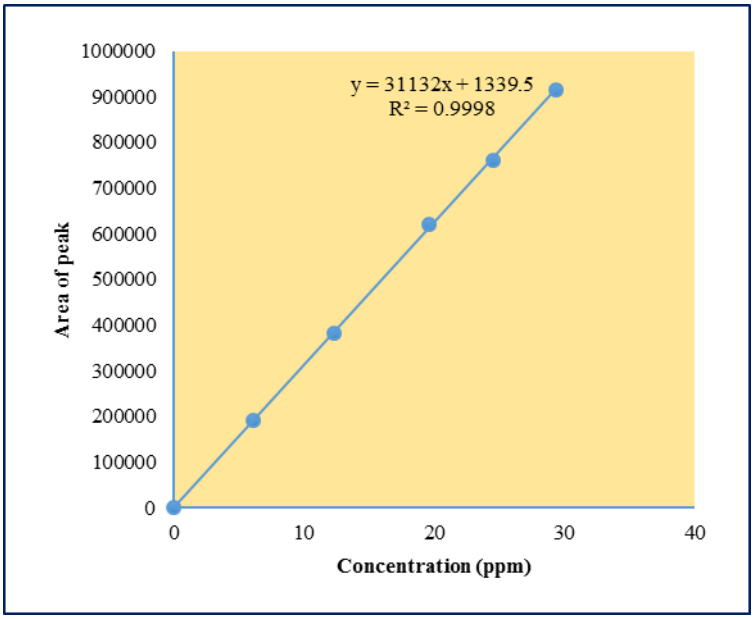

Fig. 4: Linearity plot of Galantamine Hydrobromide

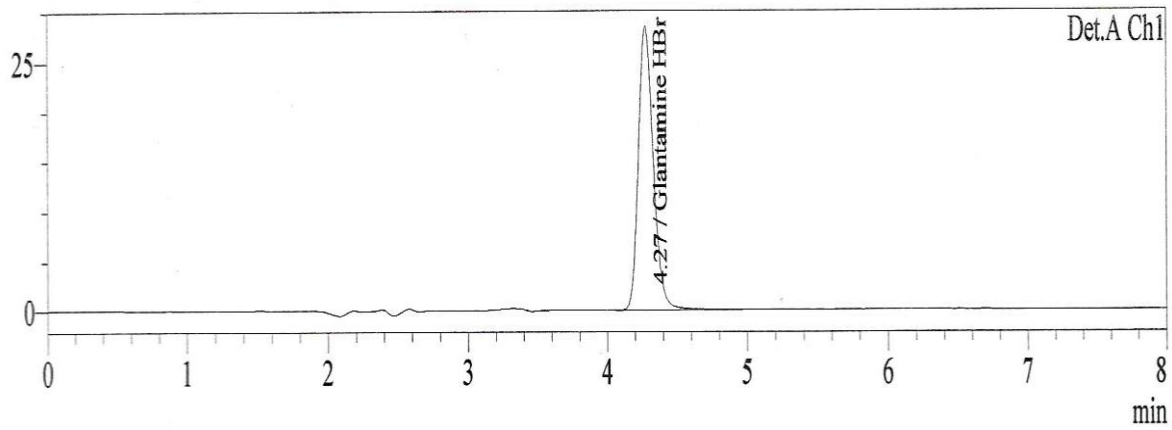

Fig. 5: A Typical Chromatogram of Galantamine Hydrobromide

REFERENCES

1. Amit VP, Vishal JP, Avani VP, Jayant BD and Chhaganbhai NP. Determination of galantamine hydrobromide in bulk drug and pharmaceutical dosage form by spectrofluorimetry. J Pharm Bioallied Sci. 2013;5(4):314–317.
2. Elif OS and Tatar U. Highly sensitive HPLC method for the determination of galantamine in human plasma and urine through derivatization with dansyl chloride using fluorescence detector. J Biolo Chem Lumines. 2017;32(7):1145-1149.
3. Tsvetkova DD and Obreshkova DP. Validation of HPLC method for simultaneous determination of galantamine Hydrobromide and pymadine. IJPRAC. 2018;7(3):233-47.
4. Lohan S, Kaur R, Bharti S, Mehta SK and Singh B. QbD-enabled development and validation of a liquid chromatographic method for estimating galantamine hydrobromide in biological fluids. Current Pharma Anal. 2018;14(6):527-40.
5. Amol UG, Karunakar S and Sourabh J. Simple HPLC method development for the estimation of galantamine hydrobromide in extended-release formulation. JPRI. 2021;33(45):225-240.