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Quantitative determination of Galantamine Hydrobromide in pharmaceutical dosage form by RP- High Performance Liquid Chromatography

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Abstract

A simple, accurate and precise validated RP-HPLC method for determination of Galantamine Hydrobromide has been developed. Analysis was carried out on Shimadzu HPLC system with Phenomenex C_{18} column (250 x 4.6 mm i.d, 5µm particle size) using 1 mM ammonium formate: acetonitrile (30:70) in Isocratic mode as mobile phase with flow rate of 0.4 $ml.min^{-1}$. The detection was carried out using UV detector set at 289 nm. For this method, Beer's law is obeyed in the concentration range of 100 to 1000 µg mL^{-1} of Galantamin Hydrobromide. The developed method has been successfully applied for the analysis of drug in bulk and pharmaceutical formulations. The mean percent recoveries were found to be 99.8 ± 0.13 for Brand formulation. The method was validated with respect to linearity, precision and accuracy as per the International Conference on Harmonisation (ICH) guidelines.

Keywords: Galantamine Hydrobromide, RP-HPLC.

Introduction [1-4]

Galantamine Hydrobromide is a reversible, competitive acetyl cholinesterase inhibitor. It is known chemically as (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. It has an empirical formula of $C_{17}H_{21}NO_3$ HBr and a molecular weight of 368.27. Galantamine hydrobromide is indicated for the treatment of mild to moderate dementia of the Alzheimer's type. Galantamine hydrobromide is a white to almost white powder and is sparingly soluble in water. The structural formula for galantamine hydrobromide is shown in figure-1.

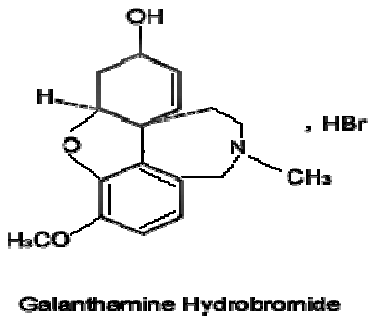

Figure 1: Chemical Structure of Galantamine Hydrobromide

There is no any official method for determination of Galantamine Hydrobromide. Different analytical methods like, HPLC [1], micellar electrokinetic chromatography–electrospray ionization mass spectrometry [2], high-performance liquid chromatographic method with UV photodiode-array, fluorescence and mass spectrometric detection [3], RP-HPLC [4] and liquid chromatographic–tandem mass spectrometric method [5] were reported for determination of Galantamine Hydrobromide in biological fluids as well as in plants. Although these techniques are sufficiently sensitive, most of them use expensive instruments or are somewhat tedious and time-consuming.

The non-availability of High-Performance Liquid Chromatography method until now for the analysis of this component made it worthwhile objective to pursue the present research work. Therefore, in the proposed work, a successful attempt has been made to develop analytical method with due consideration of accuracy, sensitivity, rapidity, economy.
The method was validated as per ICH guideline. [12]

Materials and Methods

2. Experimental

2.1. Instrument and Condition

HPLC system: Shimadzu LCVP 2010C integrated system equipped with quaternary gradient pump, 2010C UV-Vis detector, and 2010C-column oven and 2010C programmable auto sampler controlled by CLASS-VP software.
Chromatographic parameters:

- **Column-** Phenomenex C_{18} (250 X 4.6 mm), 5 µm
- **Detector-** UV-Visible.
- **Wavelength-** 289nm,
- **Flow rate-** 0.4 mL min^{-1}.
- **Mobile phase-** 1 mM ammonium formate: Acetonitrile (30:70) in isocratic mode
- 1 mM ammonium formate - Ammonium formate buffer (1 mM) was prepared by dissolving accurately about 0.01577 gm of ammonium formate in 500 ml of triple distilled water in 500 ml volumetric flask.
- **Diluent –** acetonitrile

2.2. Reagents

Galantamine Hydrobromide reference standard. - Assigned purity, 99.5% (Cadila Healthcare Ltd, Gujarat, India).

Acetonitrile. – AR grade (Spectrochem).
Ammonium formate – AR grade, E-Merck Limited.
Galamer 4 mg tablet - Claimed to contain 4 mg of the drug. Procured from the Sun pharmaceuticals ltd, Gujarat, India.

2.3. Standard Solution

Standard Galantamine Hydrobromide (100 mg) was accurately weighed and transferred to 100 ml volumetric flask. It was sonicated to dissolve properly and diluted up to mark with triple distilled water to obtain final concentration of 1000 µg/ml. Now 5 ml of this solution was further diluted to get final concentration of 500 µg/ml and used as standard solution.

2.4. Sample preparation

The contents of 20 tablets were weighed and their mean weight determined and finely powdered. An equivalent weight of the tablet content (50 mg) was transferred into a 50 ml volumetric flask containing 20 ml triple distilled water, sonicated for 30 min and further diluted to 50 ml with triple distilled water. The resulting solution was sonicated for 30 min and supernatant was filtered through whatman filter paper. 3 ml from this solution was transferred to 10 ml volumetric flask and diluted with triple distilled water to get 300 µg/ml concentration.

Results and Discussion

The detection wavelength of 289 nm was chosen in order to achieve a good sensitivity for quantitative determination Galantamine Hydrobromide in tablet dosage. The mobile phase consisting of 1 mM ammonium formate: Acetonitrile (30:70) in isocratic mode offered a good separation at ambient temperature under these conditions using a flow rate of 0.4 mL min^{-1} and a runtime of 10 min, shown in the chromatogram, Fig. 1(b), (c), (d) and (e), which illustrate the separation of active ingredient in this system. The isocratic program throughout HPLC method was adopted to analyze Galantamine Hydrobromide with greater accuracy. The proposed method is simple and do not involve laborious time-consuming sample preparation.

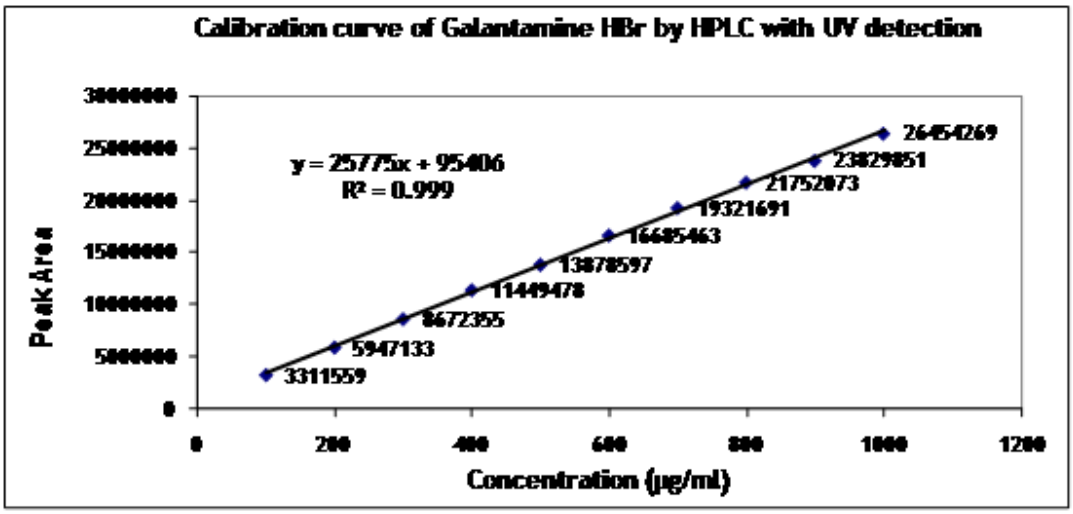

Figure 2. Calibration curve of Galantamine Hydrobromide

3.1. Linearity and calibration curve:

The plot of peak area response against concentration is shown in Fig. 1 (C). The plot is linear over the concentration range of 20 to 80 µg mL-1 of Galantamine Hydrobromide respectively. Linearity of the calibration curve was determined by weighed (1/c) least square regression analysis. The correlation coefficient was found to be 0.999. A linear relationship was found for all components.

Table 1. Characteristics of the analytical method derived from the standard calibration curve

Compound	LOD	LOQ	Linearity Range µg/mL	Linearity Equation	Correlation Coefficient	Slope of curve
Galantamine Hydrobromide	6.48	19.65	20-80	y = 43117x + 2581	0.999	2581

3.2. System suitability

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability were presented in Table 2.

Table 2. System suitability and system precision

Compound	Retention Time	n	R	T	k’
Galantamine Hydrobromide	11.447 ± 0.0618	10263	0	1.1	153.4
Isomer-C	12.123 ± 0.0667	27187.5	1.54	1.07	33.9

n= Theoretical plates
k’= Capacity Factor
R= Resolution
T= Asymmetry

Table 3. Method precision

Compound	Concentration µg/mL	Retention time Mean ± SEM	% Assay Mean ±SEM (n=6)	% RSD of Assay (n=6)
Galantamine Hydrobromide	40	11.623±0.0108	98.45±0.5857	0.59
Isomer-C	40	12.354±0.0131		0.59

3.3. Method Precision

The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in Table 3.

3.4. Method accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were presented in Table 4.

Table 4. Method accuracy

Compound	Level	Drug Added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
Galantamine Hydrobromide	50%	10.01	9.84	98.4±0.356	0.36
	100%	10.03	9.86	99.1±0.125	0.51
	150%	10.05	9.88	99.5±0.541	0.24

3.5. Method robustness

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

Table 5. Method robustness (% RSD) in Normal and Changed condition (n=5)

Compound	Condition	Change	%RSD
Sirolimus	Temperature	Normal	0.13
		−5°C	0.16
		5°C	0.17
	Flow Rate	Normal	0.13
		-10%	0.22
		10%	0.17
	Mobile Phase Ratio	Normal	0.13
		- 2%	0.14
		2%	0.16

3.6. Method Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in Table 6.

Table 6. Method ruggedness

Condition	% Assay (n=6)	% RSD of Assay Mean± SEM (n=6)
Day 1	Analyst-1, Instrument-1 & Column-1	
	99.1±0.125	0.36
Day 2	Analyst-2, Instrument-2 & Column-2	
	98.5±0.261	0.52

3.7. Specificity

There was no interference from sample placebo and peak purity of Galantamine Hydrobromide and Isomer-C were 0.9998 and 0.9997, respectively. It showed that developed analytical method was specific for the analysis of Galantamine Hydrobromide in tablet dosage form.

3.8. Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 18 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 18 h at room temperature.

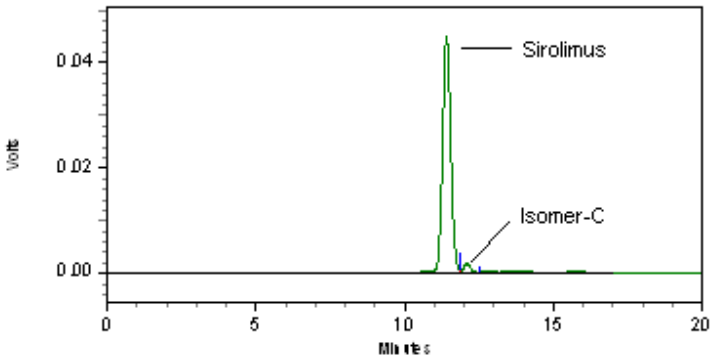

Fig. 1(b) Chromatogram of Galantamin Hydrobromide API

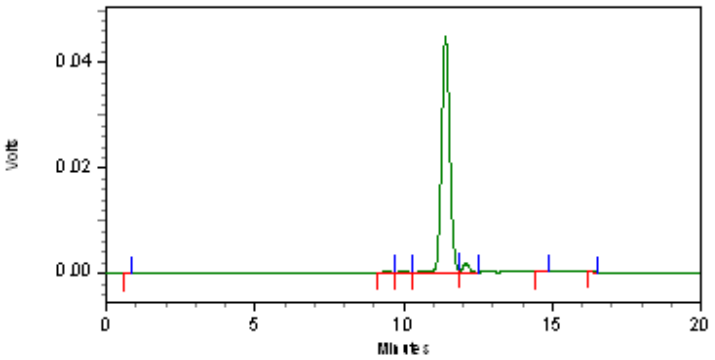

Fig. 1(c) Chromatogram of Galantamin Hydrobromide Tablet Solution

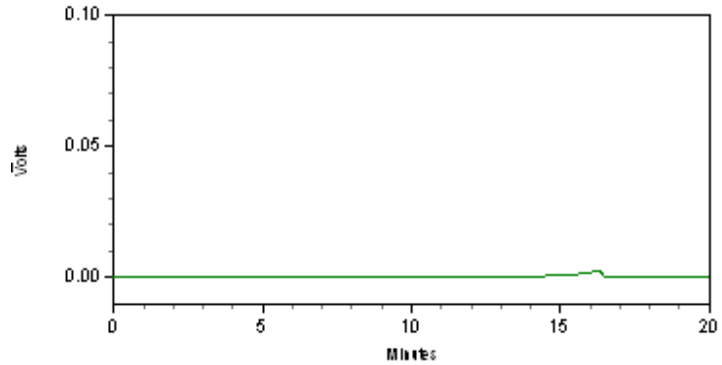

Fig. 1(d) Chromatogram Diluent-Acetonitrile

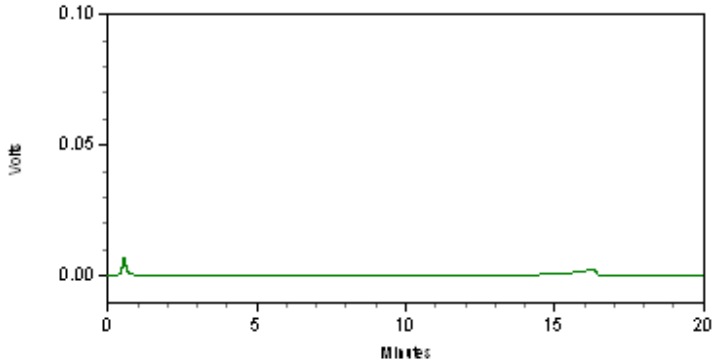

Fig. 1(e) Chromatogram of Placebo of Galantamin Hydrobromide

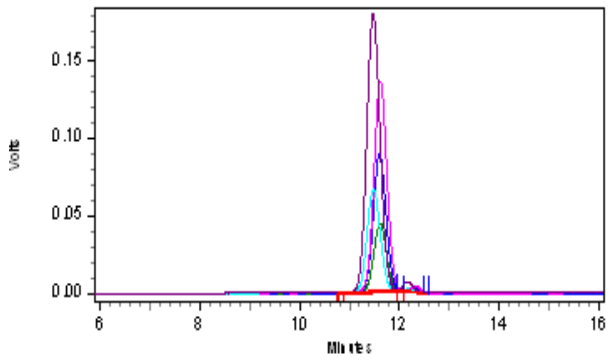

Fig. 1(f) Chromatogram of Linearity Solutions

Conclusion

The method described enables to the quantification of Galantamine Hydrobromide. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of

satisfactory precision and reproducibility. Hence, this HPLC method can be used for routine drug analysis.

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