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Simple HPLC Method Development for the Estimation of Galantamine Hydrobromide in Extended-Release Formulation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

For the determination of Galantamine Hydrobromide in bulk and produced extended formulation, a new sensitive and quick HPLC technique was developed and validated according to ICH guidelines. The HPLC analysis was carried out using a waters system with a Thermo Scientific C18 (steel column (5 µm, 250mm × 4.6 mm)) column and a mobile phase of 0.1M phosphate buffer: Acetonitrile (40:60V/V) pH adjusted to 4.5 with orthophosphoric acid, at a flow rate of 1.0 mL/min. The detection was done at a wavelength of 203 nm, and galantamine hydrobromide had a retention time of 8.0 minutes. Over the concentration range of 1-10 g/ml, the calibration plot revealed a linear relationship. The accuracy of the proposed method was determined by recovery studies and was found to be near to 100 and % RSD value was found less than 2. The repeatability testing for both standard and sample solutions showed that the method is precise within the acceptable limits. RSD % of the determination of precision was <2%. The proposed method showed excellent linearity, accuracy, precision, specificity, robustness and system suitability results within the acceptance criteria. In addition, Saturation solubility of Galantamine Hydrobromide was determined in different pH mediums and it was found that Galantamine Hydrobromide has pH-dependent solubility, freely soluble in alkaline pH, and insoluble in acidic pH.

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Keywords: HPLC; robustness; galantamine hydrobromide; validation.

1. INTRODUCTION

Extended-release systems provide drug release in an amount sufficient to maintain the therapeutic drug level over an extended period, with the release profiles predominantly controlled by the special technological construction and design of the system itself. The development of oral extended-release systems has been a challenge to formulation scientists due to their inability to restrain and localize the system at targeted areas of the gastrointestinal tract. There are numerous products in the market formulated for both oral and parenteral routes of administration that claim extended or controlled drug delivery. Matrix-type drug delivery systems are one of the interesting and promising options in developing an oral extended-release system. In particular, the interest awakened by matrix type delivery is completely justified because of its biopharmaceutical and pharmacokinetic advantages over the conventional dosage forms [1-5].

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. This idealized objective of delivering drug at a rate dictated by the needs of the body throughout treatment points to the two aspects most important to drug delivery, namely, spatial placement and temporal delivery of a drug. Spatial placement relates to targeting drug delivery to the target tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. Despite the significant interest and numerous reports about the design of extended delivery systems for various types of drugs, very few have been successful [6-7].

Galantamine Hydrobromide is the hydrobromide salt form of galantamine, a tertiary alkaloid obtained synthetically or naturally from the bulbs and flowers of Narcissus and several other genera of the Amaryllidaceae family with anticholinesterase and neurocognitive-enhancing activities. Galantamine competitively and reversibly inhibits acetylcholinesterase, thereby increasing the concentration and enhancing the action of acetylcholine (Ach). In addition, galantamine is a ligand for nicotinic acetylcholine receptors, which may increase the presynaptic release of Ach and activate postsynaptic receptors. This agent may improve neurocognitive function in mild and moderate Alzheimer' s disease and may reduce abstinence-induced cognitive symptoms that promote smoking relapse [7-11].

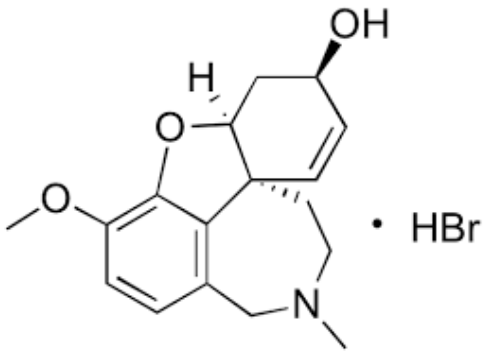

Fig. 1. Chemical Structure of Galantamine Hydrobromide

2. MATERIALS AND METHODS

2.1 Development of Analytical Methods

HPLC method for analysis of Galantamine Hydrobromide was developed. As the method was supposed to be used for the analysis of dissolution samples and stability samples, the drug was subjected to stress conditions and analyzed by the HPLC method. Good separation and resolution of the drug and degradation products ensure that the method can be successfully used for stability analysis.

2.2 Chromatographic Conditions

Instrument:	Jasco HPLC with PDA detector
Column:	A stainless steel column (5 µm, 250mm × 4.6 mm) packed with particles of silica C18 Reverse phase column, LICROSPHER® 100 RP-18e
Flow rate:	1 ml per minute
Wavelength:	203 nm Injection volume: 100 ml
Mobile phase:	0.1M phosphate buffer: Acetonitrile (40:60V/V) pH adjusted to 4.5 with orthophosphoric acid
Diluent:	Mobile phase
Run time:	7 minutes

Temperature: Ambient

2.3 Preparation of Solutions

2.3.1 Preparation of buffer solution: (mixed buffer)

Potassium dihydrogen orthophosphate (3.4 gm) and dipotassium hydrogen orthophosphate (4.36 gm) were dissolved in a 1000 ml volumetric flask containing 500 ml of HPLC grade water. The final volume was made with HPLC-grade water. The solution was filtered with 0.45-micron membrane filter paper.

2.3.2 Preparation of mobile phase

In a volumetric flask of 200 mL, 80 ml of mixed buffer solution and 120 ml of acetonitrile were added. The solution was mixed well and sonicated for 10 min. The pH of the mobile phase was adjusted to 4.5 with orthophosphoric acid.

2.3.4 Preparation of stock solution

25 mg Galantamine Hydrobromide standard was accurately weighed and was transferred into a volumetric flask of 50 ml. Methanol (40 mL) was added to the flask and the solution was sonicated for 15 min. The volume was made up to 50 mL with methanol. This solution (1mL) was further diluted up to 50 mL with mobile phase to give a final solution of 10 µg/mL.

2.3.5 Stress testing

Stress testing helps to analyze the intrinsic stability characteristics of the drug molecule. It provides data on properties like degradation pathways, leads to the identification of degradation products, and helps in developing the stability-indicating HPLC assay method. Galantamine Hydrobromide was subjected to various stress conditions as mentioned below procedure.

2.3.6 Acid degradation

The drug was subjected to forced degradation under acidic conditions (2N HCl). Galantamine Hydrobromide (25 mg) was weighed accurately and was transferred to a volumetric flask of 50 mL containing 20 mL mobile phase. 2ml of acid solution (2N) was added to it and heated at 60°C for 3 hrs in the water bath. The solution was then further cooled to room temperature and was neutralized by using 2N sodium hydroxide solution to adjust it to pH 7. Volume was made up to 50mL with mobile phase to get 500 mg/mL solution of Galantamine Hydrobromide. This solution (1 mL) was further diluted up to 50 mL with mobile phase to give 10 mg/mL solution of Galantamine Hydrobromide and was injected to chromatography to obtain chromatogram.

2.3.7 Acceptance criteria

Interference should not be observed at retention time (RT) of Galantamine Hydrobromide.

2.3.8 Base degradation

The drug was subjected to forced degradation under basic conditions (2N NaOH). Galantamine Hydrobromide (25 mg) was weighed accurately and was transferred to a 50 mL volumetric flask containing a 20 mL mobile phase. 2ml of base solution (2N) was added to it and heated at 60°C for 3 hrs in a water bath. This solution was cooled to room temperature and was neutralized by using 2N HCl solution to pH 7. Volume was made up to 50mL with mobile phase to get 500 mg/mL solution of Galantamine Hydrobromide. This solution (1 mL) was further diluted up to 50 mL with mobile phase to give 10 mg/mL solution of Galantamine Hydrobromide and was injected to chromatography to obtain chromatogram.

2.3.9 Acceptance criteria

Interference should not be observed at retention time (RT) of Galantamine Hydrobromide.

2.3.10 Peroxide degradation

The drug was subjected to forced degradation under oxidation (30% v/v H2O2 solution). Galantamine Hydrobromide (25 mg) was weighed accurately and was transferred to a 50 mL volumetric flask containing a 20 mL mobile phase. 5mL of (30% v/v H2O2 solution) was added to it and heated at 70°C for 10 minutes in a water bath. This solution was neutralized with sodium meta bisulfate solution and volume was made up to 50 mL with mobile phase to get 500 mg/mL solution of Galantamine Hydrobromide. This solution (1 mL) was further diluted up to 50 mL with mobile phase to get 10 mg/mL solution of Galantamine Hydrobromide and was injected to obtain chromatogram.

2.3.11 Acceptance criteria

Interference should not be observed at retention time (RT) of Galantamine Hydrobromide.

2.4 Assay of Galantamine Hydrobromide Extended-release Pellets

2.4.1 Preparation of test solution

Capsules (20) were randomly sampled and were emptied in mortar and pestle. The pellets were crushed to a fine powder. Powder equivalent to 5 mg of Galantamine Hydrobromide was weighed accurately and was transferred to a 50 mL volumetric flask. About 40 mL of methanol was added and the solution was sonicated for 15 minutes. The volume was made up to 50 ml with mobile phase and the solution was mixed. The solution was filtered through a 0.45µ membrane filter, about 2mL of the initial filtrate was discarded and the remaining filtrate was collected. This solution (1 mL) was further diluted to 10 mL with mobile phase and was injected into chromatography.

2.4.2 Preparation of the standard solution

Galantamine Hydrobromide (25 mg) was weighed accurately and transferred into a 50 mL volumetric flask. About 40 mL methanol was added to the flask and was sonicated for about 15 minutes to dissolve the content. Volume was made up to 50 ml with methanol. This solution (1 mL) was further diluted to 50 mL with mobile phase and was injected into chromatography.

2.4.3 Procedure

After achieving equilibration of the column using mobile phase, 100 µl of the reference solution and 100 µl of the test solution were successively injected. Chromatographic profiles were recorded.

2.4.4 Calculations

$$\%\,\text{Assay} = \frac{S_1}{50} \times \frac{S_3}{50} \times \frac{1}{50} \times \frac{S_4}{50} \times \frac{1}{10} \times \frac{S_5}{LC} \times PS_2$$

Where,

S1: ean Area of Galantamine Hydrobromide peak in test solution chromatogram

S2: Mean Area of Galantamine Hydrobromide peak in the standard solution chromatogram

S3: Standard weight (mg)S4- Sample weight (mg)

S5: Average weight of capsule (mg)
LC: Label claim
P: Purity of standard

2.4.5 Validation of developed analytical methods

The purpose of the study was to validate the test procedure for the determination of Galantamine Hydrobromide in the formulation to ensure accurate and reliable results during the finished product analysis and stability testing. Chromatographic conditions finalized during method development were used.

2.4.6 Specificity

The following injections were injected – mobile phase, placebo, reference standard (10µg/mL), and placebo plus reference standard (10µ g/mL).

2.4.7 Acceptance criteria

There should not be interference of any inactive ingredient from the placebo.

2.4.8 System suitability Standard solution preparation

Six injections of standard solution were injected into the chromatographic system and chromatograms were recorded.

2.4.9 Acceptance criteria

The % Relative Standard Deviation (RSD) of peak area and retention times of six injections should not be more than 2 %.

2.5 Linearity

2.5.1 Linearity curve for the assay

Linearity was performed on standard solution at 5 levels – 80 %, 90 %, 100 %, 110 %, and 120 % of the working concentration of Galantamine Hydrobromide (10 µg/mL). Each level was injected in triplicate to the chromatographic system and the chromatograms were thus recorded.

2.5.2 Procedure Stock Solution

An accurately weighed quantity of 50 mg Galantamine Hydrobromide standard was transferred into a volumetric flask of 100 ml. The solution was dissolved and then diluted to 100 ml

with mobile phase to give a final concentration of 500 µg/mL.

2.5.3 80 % level

Stock solution (0.8 ml) was pipetted into a 50 ml volumetric flask and volume was made up to 50 ml with mobile phase to get 8 µg/mL of Galantamine Hydrobromide.

2.5.4 90 % level

Stock solution (0.9 ml) was pipetted into a 50 ml volumetric flask and diluted with mobile phase to 50 mL to get 9 µg/mL of Galantamine Hydrobromide.

2.5.6 100 % level

Stock solution (1 ml) was pipetted into a 50 ml volumetric flask and diluted with mobile phase to 50 mL to get 10 µg/mL of Galantamine Hydrobromide.

2.5.7 110 % level

Stock solution (1.1 ml) was pipetted into a 50 ml volumetric flask and diluted with mobile phase to 50 ml to get 11 µg/mL of Galantamine Hydrobromide.

2.5.8 120 % level

Stock solution (1.2 ml) was pipetted into a 50 ml volumetric flask and diluted with mobile phase to 50 ml to get 12 µg/mL of Galantamine Hydrobromide.
A linearity curve was plotted of Galantamine Hydrobromide of the standard concentration versus area of the peak.

2.6 Acceptance Criteria

There should be a linear response in the operating range and the correlation coefficient should not be less than 0.995.

2.7 Linearity Curve of Dissolution Study

Linearity was performed on a standard solution in pH 1.2 simulated gastric fluid (SGF) (without enzymes) at 6 levels – 1, 2, 4, 8, and 10 µg/mL of the Galantamine Hydrobromide. Each level was injected in triplicate to the chromatographic system and the chromatogram was recorded.

2.8 Procedure Stock Solution

An accurately weighed quantity of 50 mg Galantamine Hydrobromide standard was transferred into a 100 ml volumetric flask. Mobile phase (80 mL) was added to the flask and solution was sonicated for 5 min. This solution (1 mL) was further diluted up to 50 mL with pH 1.2 SGF (without enzymes) to give a final solution of 10 µg/mL.

2.8.1 1 µg/mL solution

Stock solution (l.0 mL) was pipetted into a 10 ml volumetric flask and volume was made up to 10 ml with pH 1.2 SGF (without enzymes) to give 1.0 µg/mL of Galantamine Hydrobromide.

2.8.2 2 µg/mL solution

Stock solution (2ml) was pipetted into a 10 ml volumetric flask and volume was made up to 10 ml with pH 1.2 SGF (without enzymes) to give 2.0 ppm of Galantamine Hydrobromide.

2.8.3 4 µg/mL solution

Stock solution (4ml) was pipetted into a 10 ml volumetric flask and volume was made up to 10 ml with pH 1.2 SGF (without enzymes) to give 4.0 ppm of Galantamine Hydrobromide.

2.8.4 8 µg/mL solution

Stock solution (8ml) was pipetted into a 10 ml volumetric flask and volume was made up to 10 ml with pH 1.2 SGF (without enzymes) to give 8.0 ppm of Galantamine Hydrobromide.

2.8.5 10 µg/mL solution

The stock solution was directly injected into chromatography.
The linearity curve was plotted with standard concentration versus peak area.

2.9 Acceptance Criteria

There should be a linear response in the operating range and the correlation coefficient should not be less than 0.995.

2.10 Accuracy

To validate, that the test method can accurately quantify Galantamine Hydrobromide within the extended-release formulation, three samples were prepared, each by spiking Galantamine Hydrobromide raw material to an equivalent

amount of placebo at 80%, 100%, and 120% of the working concentration (10µg/ml of Galantamine Hydrobromide). Each level was weighed thrice and injected. Percentage Recovery (% Assay) for the drug was calculated for each level.

2.11 Standard Solution

Six injections of the standard solution were injected into the chromatographic system and the chromatograms were recorded.

2.11.1 800 % level

A placebo powder of Galantamine Hydrobromide osmotic pellets (168 mg) and 5 mg Galantamine Hydrobromide standard were weighed accurately. Weighed placebo and drug was added in 50 ml volumetric flask. Mobile phase (40 mL) was added to volumetric flask and solution was sonicated for 20 min. The volume was made up to 50 ml with the mobile phase. The solution was filtered through a 0.45-micron membrane filter. The solution (0.8 mL) was further diluted to 10 mL with a mobile phase to give a final concentration of 8 ppm.

2.11.2 100 % level

A placebo powder of Galantamine Hydrobromide osmotic pellets (168 mg) and 5 mg Galantamine Hydrobromide standard were weighed accurately. Weighed placebo and drug was added in 50 ml volumetric flask. Mobile phase (40 mL) was added to volumetric flask and solution was sonicated for 20 min. The volume was made up to 50 ml with the mobile phase. The solution was filtered through a 0.45-micron membrane filter. The solution (1 mL) was further diluted to 10 mL with a mobile phase to give a final concentration of 10 ppm.

2.11.3 120 % level

A placebo powder of Galantamine Hydrobromide osmotic pellets (168 mg) and 5 mg Galantamine Hydrobromide standard were weighed accurately. Weighed placebo and drug was added in 50 ml volumetric flask. Mobile phase (40 mL) was added to volumetric flask and solution was sonicated for 20 min. The volume was made up to 50 ml with the mobile phase. The solution was filtered through a 0.45-micron membrane filter. The solution (1.2 mL) was further diluted to 10 mL with a mobile phase to give a final concentration of 12 ppm.

2.12 Acceptance Criteria

Percent recovery should be between 98 % and 102 % and the RSD of percent recovery of Galantamine Hydrobromide should not be more than 2%.

2.13 Precision

2.13.1 System precision

Standard solution at a working concentration of 10 µg/ml was injected six times.

2.13.2 Acceptance criteria

The RSD of the peak area of the six injections should not be more than 2%.

2.13.3 Inter-day precision

A variability test was conducted on the HPLC system by a different analyst on a different day as per the test method. The standard solution at a working concentration (10µg/ml) was to be injected three times. The procedure was repeated on Day 2 by Chemist 2.

2.13.4 Acceptance criteria

The RSD of the % area values of the injections should not be more than 2%. The difference in the area values on Day 1 should not be more than 3% from Day 2.

2.14 Robustness

2.14.1 Standard solution (Normal conditions) Preparation of standard solution

Six injections of standard solution were injected into the chromatographic system and the chromatograms were recorded.

2.14.2 Preparation of the sample solution

Three injections of sample solution were injected into the chromatographic system to record the chromatograms. The percent assay of each sample solution was calculated.

2.14.3 Acceptance criteria

The % relative standard deviation of peak area, the retention time of three standard solutions, the percent assay, and retention times of two sample injections should not be more than 2%.

3. RESULTS AND DISCUSSION

3.1 Development of Analytical Methods

3.1.1 Stress testing

Galantamine hydrobromide and its degradation products (impurities) were separated on a stainless steel Supelcosil LC8, 5µ, 250 mm x 4.6 mm, column. The degradation products are shown inthe following chromatograms.

The galantamine hydrobromide peak is well resolved from galantamine hydrobromide impurity-A and impurity-C. The resolution between galantamine hydrobromide peak and impurity-A peak was greater than 2.5.

Table 1. Optimized chromatographic conditions for assay of Galantamine Hydrobromide

Column	LICROSPHER® 100 RP-18e (5 µm, 250 mm x 4.6 mm)
Mobile phase	0.1M phosphate buffer: Acetonitrile (40:60V/V) pH adjusted to 4.5 with orthophosphoric acid
Diluting solvent	Mobile phase
Flow rate	1.0 ml
Wavelength	203nm
Injection volume	100 µL
Run time	7 min
Method	Isocratic method

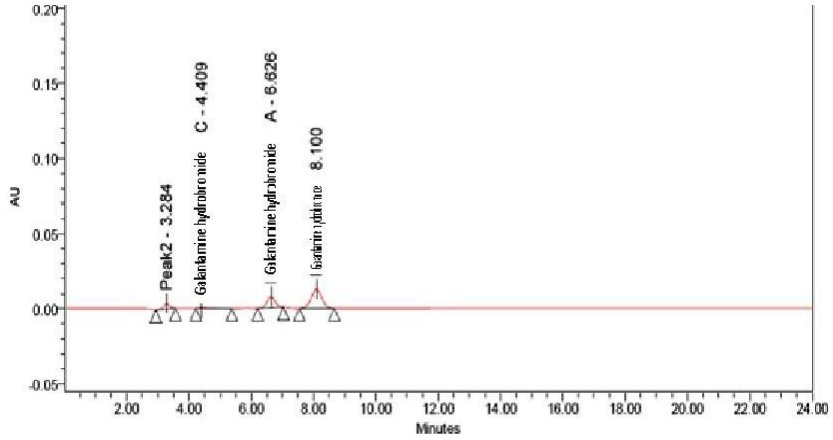

Fig. 2. Chromatogram of Galantamine hydrobromide and their impurities

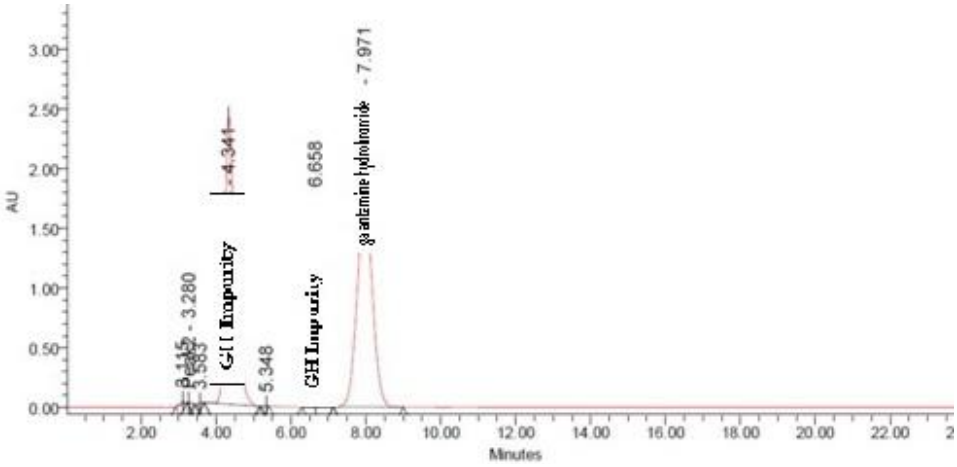

Fig. 3. Chromatogram of acid degradation

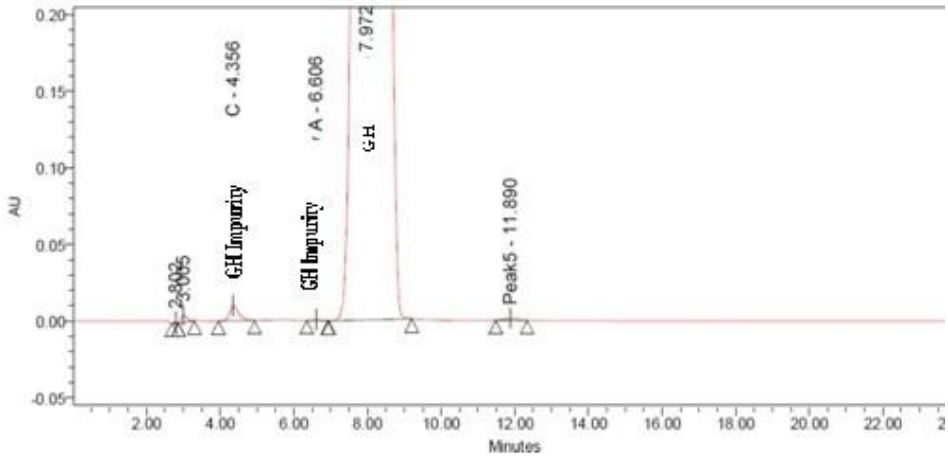

Fig. 4. Chromatogram of base degradation

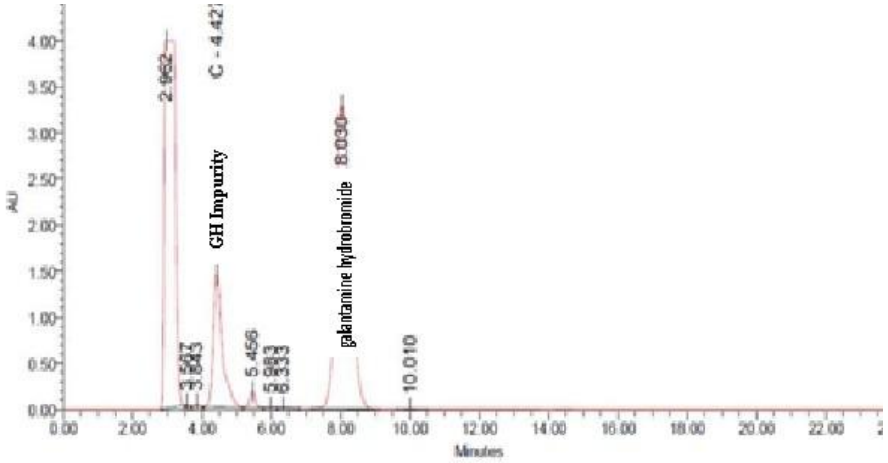

Fig. 5. Chromatogram of peroxide degradation

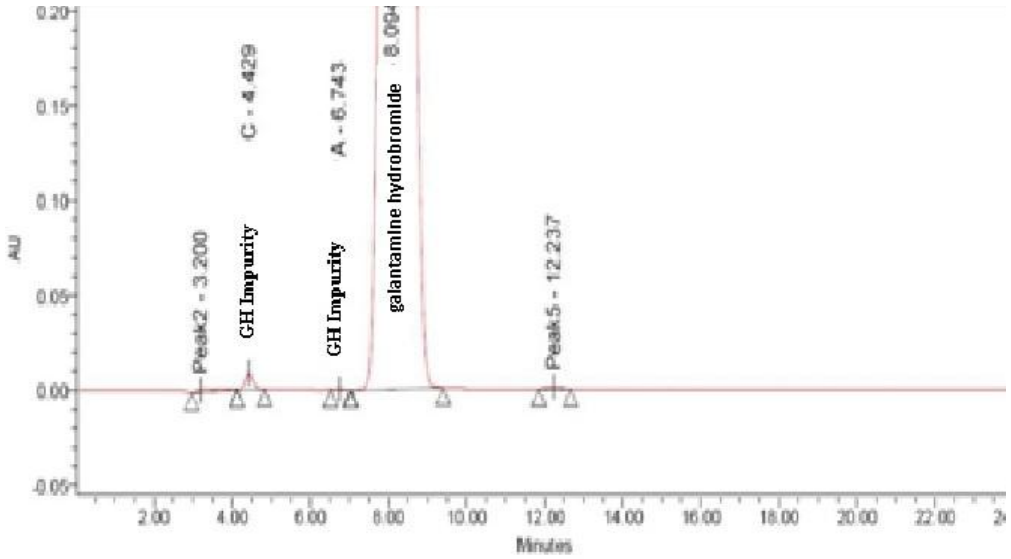

Fig. 6. Chromatogram of photo degradation

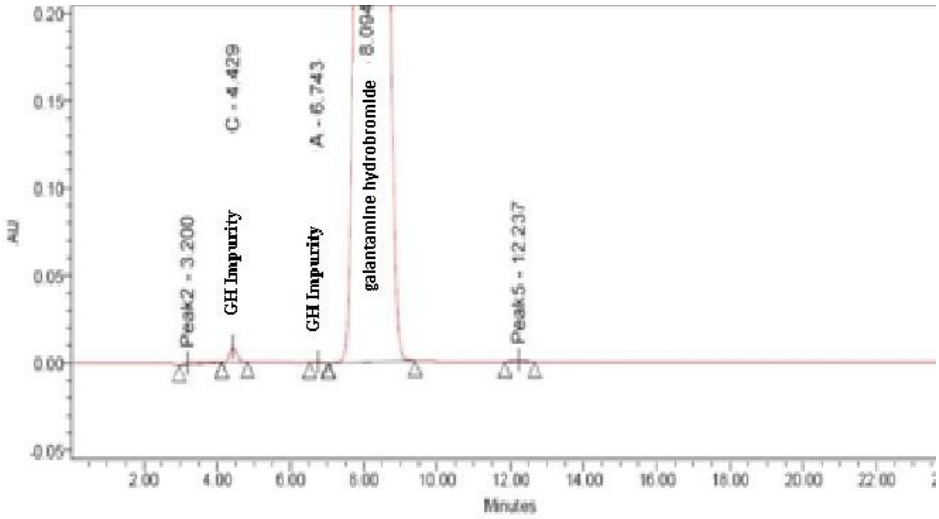

Fig. 7. Chromatogram of solid-state heat degradation

Table 2. Retention time of Galantamine hydrobromide and its degradation product (Impurities)

Sample	Retention Time (min)	Relative Retention Time
Galantamine hydrobromide	8.0	--
Galantamine hydrobromide impurity A	6.65	0.83
Galantamine hydrobromide impurity C	4.34	0.54
Unknown impurity 1	12.01	1.5

Table 3. Peak area of Galantamine hydrobromide standard and sample

S. No	Peak Area	Mean Area	RSD
1	7963202		
2	7965961	7966218	0.04%
3	7969492		

Injection	Sample area	Standard area	% Assay
1	7990023	7966218	100.46
2	7994479	7966218	100.51
3	8005443	7966218	100.65

Acid degradation solution was prepared as per proposed method and the solution was injected to the chromatographic system. There was no degradation peak interfering with galantamine hydrobromide peak. The degraded peaks were well separated from galantamine hydrobromide peak.

Base degradation solution was prepared as per proposed method and the solution was injected to the chromatographic system. There was no degradation peak interfering with galantamine hydrobromide peak. The degraded peaks were well separated from galantamine hydrobromide peak.

Peroxide degradation solution was prepared as per proposed method and the solution was injected to the chromatographic system. There was no degradation peak interfering with galantamine hydrobromide peak. The degraded peaks were well separated from galantamine hydrobromide peak.

The galantamine hydrobromide was exposed to UV light as per proposed method and the solution was injected to the chromatographic system. There was no degradation peak interfering with galantamine hydrobromide peak. The degraded peaks were well separated from galantamine hydrobromide peak.

The drug was subjected to dry heat degradation as per proposed method and the solution was injected to the chromatographic system. There was no degradation peak interfering with galantamine hydrobromide peak. The degraded peaks were well separated from galantamine hydrobromide peak.

3.1.2 Assay of galantamine hydrobromide tablets

The assay of galantamine hydrobromide tablets was performed as per the method described in the experimental section.

The assay of the tablets was within the acceptable limit (between 90% and 110%).

3.1.3 Validation of developed analytical methods

3.1.4 Specificity

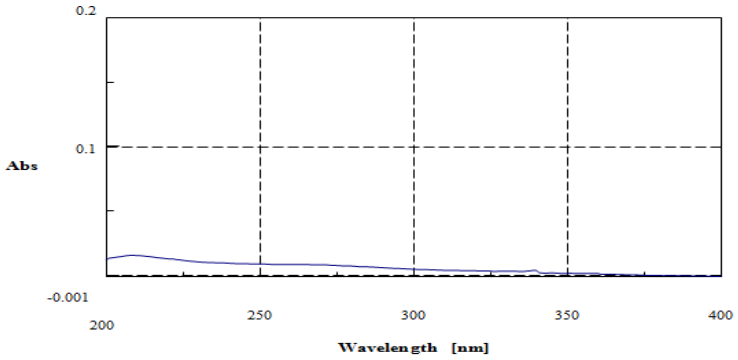

Fig. 8. Spectrum of placebo solution

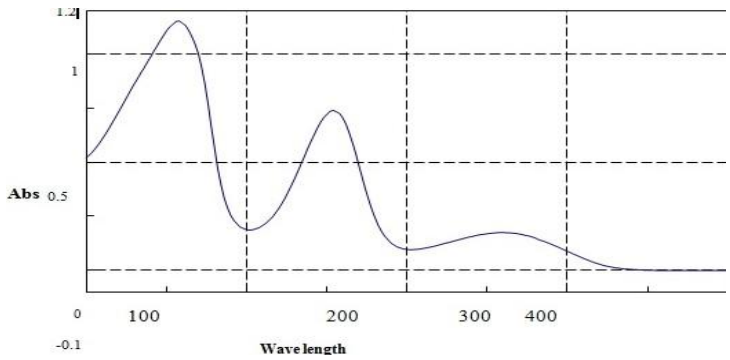

Fig. 9. Spectrum of reference solution (reference substance)

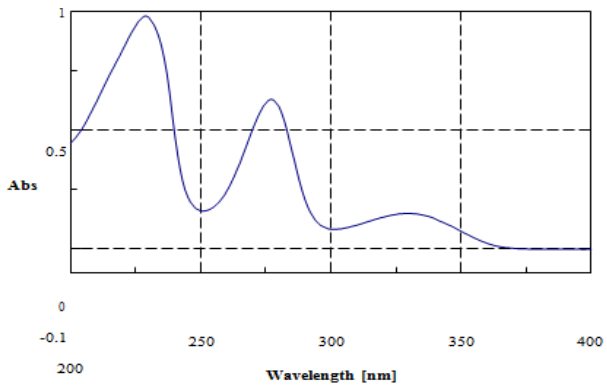

Fig. 10. Spectrum of test solution (placebo + reference substance)

3.1.5 Linearity

The polynomial regression data for calibration plots (n=3) proved a good linear relation over concentration range of 4 to 9.3 µg/mL. Coefficient of correlation (r) was 0.999 (acceptance criteria = 0.995) with slope of 0.003 and intercept of 0.52. No significant difference was observed in the slopes of standard curve. Table 4 represents the linearity of galantamine hydrobromide response.

There was a good correlation of data over the range 4.0 µg/mL to 9.3 µg/mL of galantamine hydrobromide.

Table 4. Linearity of Galantamine hydrobromide response by UV Spectroscopy

Test	Level in %	Final Concentration in µg/Ml	Response
1/1	60	4.0	0.2457
2/1	60	4.0	0.2433
3/1	60	4.0	0.2444
½	80	5.0	0.3021
2/2	80	5.0	0.3017
3/2	80	5.0	0.3027
1/3	100	6.6	0.3608
2/3	100	6.6	0.3667
3/3	100	6.6	0.3669
¼	120	8.0	0.4345
2/4	120	8.0	0.432
¾	120	8.0	0.4334
1/5	140	9.3	0.4934
2/5	140	9.3	0.4967
3/5	140	9.3	0.5008

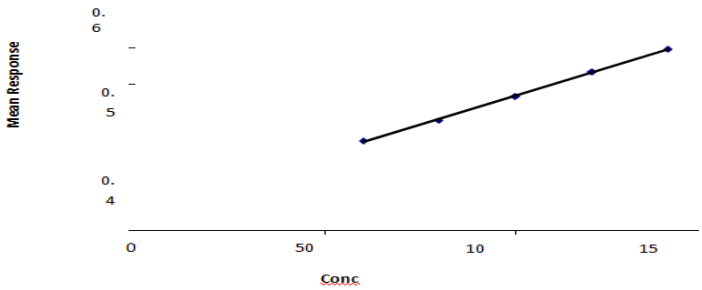

Fig. 11. Linearity curve of Galantamine hydrobromide

Correlation	0.999
Slope	0.003
Y Intercept	0.052
Straight line equation	Y= 0.003x +0.052

3.1.6 Precision

Table 5. Absorbance of Galantamine hydrobromide Standard solution for precision (n=6)

S. No	Absorbance
1	0.3798
2	0.3863
3	0.3876
4	0.3898
5	0.382
6	0.3764
RSD	1.33

Table 6. Precision: Day 1 peak area

Precision: Day 1				
S. No.	**Absorbance**	**Mean**	**STDEV**	**RSD**
1	0.3764			
2	0.3862			
3	0.3872			
4	0.3869	0.3818	0.0054	1.42
5	0.3782			
6	0.3763			

Table 7. Precision: Day 2 peak area

Precision: Day 2				
S. No.	**Absorbance**	**Mean**	**STDEV**	**RSD**
1	0.3724			
2	0.3768			
3	0.3864			
4	0.382	0.3771	0.0063	1.68
5	0.3689			
6	0.3764			

Table 8. Precision: Day 3 peak area

Precision: Day 3				
S. No.	**Absorbance**	**Mean**	**STDEV**	**RSD**
1	0.374			
2	0.3789			
3	0.3763			
4	0.3843	0.3784	0.0034	0.918
5	0.3789			
6	0.3784			

Table 9. Instrumental precision data of UV-Spectroscopic method (n=3)

S. No.	Result Day 1 (%)	Result Day 2 (%)	Result Day 3 (%)
1	97.6	96.7	96.9
2	99.2	99.2	97.4
3	99.6	99.5	98.7
4	100.1	99.4	100.0
5	98.1	97.2	99.6
6	96.7	96.7	99.3

Table 10. Precision data of repeatability of UV-Spectroscopy (n=6)

S. No.	Day 1	Day 2	Day 3
Mean	98.49	96.89	97.23
Minimum	96.7	94.8	96.1
Maximum	100.1	99.3	98.7
Standard Deviation	1.38	1.62	0.882
C V %	1.40	1.67	0.908

Table 11. Precision data of reproducibility of UV-Spectroscopic method (n=18)

Mean %	97.5
Minimum	94.8
Maximum	100.1
Standard Deviation	1.43
C V %	1.47

The result along with the percent RSD, dissolution of drug, shown in table 10, indicates an acceptable precision level for the analytical system for each day less than 2%. (Acceptance criteria: RSD ≤ 2 %).

The result along with the percent RSD, dissolution of drug, shown in table indicates an acceptable level of precision for the analytical system for each day for 18 values is less than 2%. (Acceptance criteria: RSD ≤ 2 %).

3.1.7 Accuracy

The accuracy of the method was checked by recovery of drug from dissolution mediums, accurately spiked with different concentrations of the drug. The results reported in table, confirmed that there were no significant difference between the calculated percent recovery and actual value drug (Acceptance criteria - Percent recovery should be between 98 % and 102 % and RSD of percent recovery should not be more than 2 %).

3.1.8 Robustness

The UV analysis was carried out using the method outlined in the methodology section and by making the following alterations in the dissolution conditions.

- Changing the volume of the dissolution medium (890 mL, 900 mL and 910 mL)
- Changing UV detection lamda max (272 nm, 274 nm and 276 nm)

Table 12. Galantamine hydrobromide Sample solution and Standard Solution Absorbance for accuracy

Particulars	60%	80%	100%	120%	140%	Std
	Absorbance					
	0.251	0.326	0.409	0.492	0.581	0.412
	0.249	0.331	0.413	0.495	0.575	0.409
	0.245	0.333	0.414	0.498	0.578	0.410
Mean	0.248	0.330	0.412	0.495	0.578	0.410
Max.	0.251	0.333	0.414	0.498	0.581	0.412
Min.	0.245	0.326	0.409	0.492	0.575	0.409

Table 13. Accuracy of UV-Spectroscopic method for estimation of galantamine hydrobromide

S. No	Level in %	Conc. in µg/mL	Mean Absorbance	% Recovery
1	60%	4.0	0.2483	100.6
2	80 %	5.3	0.33	100.47
3	100 %	6.6	0.4125	100.48
4	120 %	8.0	0.495	100.77
5	140 %	9.3	0.578	100.55

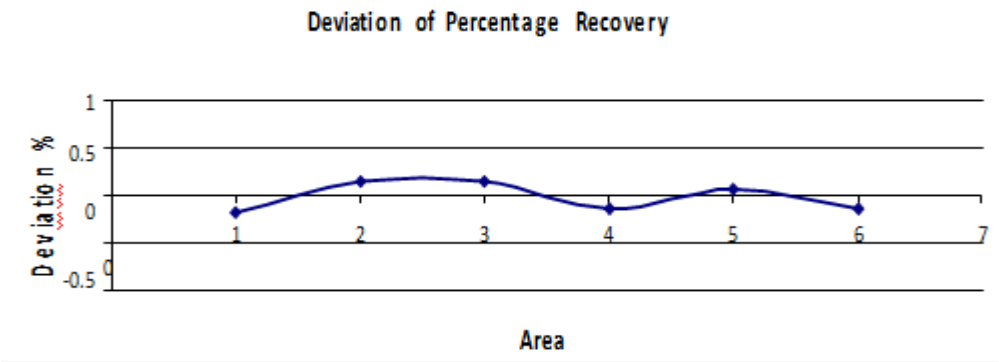

Fig. 12. Deviation of percent recovery of accuracy study

Table 14. Robustness of UV-Spectroscopic method for estimation of Galantamine hydrobromide

Altered condition	Mean absorbance	% Dissolution	Absolute Difference (w.r.t. unaltered)
Change in Volume of Dissolution Medium			
Unaltered Condition			
Dissolution Volume: 900 mL	0.3888	100.28	--
Altered Condition			
Dissolution Volume: 890 mL	0.3904	100.68	0.4
Dissolution Volume: 910 mL	0.3884	100.18	-0.1
Change in Lambda Max			
Unaltered Condition			
274 nm	0.38288	101.09	--
Altered Condition			
272 nm	0.3719	99.29	-1.79
276 nm	0.371	98.98	-2.11

The method was found to be robust in terms of small change in dissolution volume and change in UV detection lamda max as absolute difference between normal conditions and altered conditions was not more than 5.0 % (Acceptance criteria: absolute difference NMT 5.0%).

4. DISCUSSION

Analytical methods were developed for analyzing galantamine hydrobromide in bulk as well as final formulations. The calibration curves of galantamine hydrobromide were prepared in pH 1.2, pH 4.5, pH 5.8, pH 6.8, pH 7.4 systems. The linear relation was found for absorbance and concentration in a range of 2-12 µg/mL with a regression value of 0.999 indicating that the UV method could be applied for analysis during the study [12].

The HPLC method was adopted well and all the chromatograms from the stress testing showed that the retention time of the degradation peak and galantamine hydrobromide peak were well separated. There was no interference or overlapping of impurities with the galantamine hydrobromide peak thus ensuring the applicability of the method for analysis. Thus the method was planned to be applied for testing of galantamine hydrobromide and related substances in the extend release tablet formulation. Followed by the analytical method development the experiment on the assay of galantamine hydrobromide tablets yielded results which were within the acceptable limit of between 90% and 110%. Also, the results on galantamine hydrobromide content analysis in the tablets were within the acceptable limits of 85 % and 115 % per tablet [13-14].

4.1 Validation of Developed Analytical Methods

Specificity absorbance spectrum for the placebo and placebo plus reference showed a good separation peaks. Linearity over the range of 4 to 9.3 µg/mL was within the acceptance limits having an coefficient of correlation (r) to be 0.999 (acceptance limit = 0.995). With a good linearity it was also observed that the standard curves had no significant difference. Precision values achieved from the experiment for the dissolution testing of drug were less that 2% which is well within the acceptance criteria of RSD ≤ 2 %. Accuracy evaluated by recovery of drug from dissolution medium had no significant difference between the actual and calculated percent recovery of drug value. The accuracy value was within the acceptance criteria of between 98% and 102 % meaning that the percent recovery was not more than 2%. Robustness evaluated on two parameters of changing the volume of dissolution medium and UV detection lambda max was within the 5% acceptance criteria for the absolute difference between normal and altered conditions, confirming that the method was robust enough. The solution stability studies proved that the drug could be stored at room temperature for 24 hrs as the percentage dissolution difference in sample solution was within the acceptance criteria of less than 2%. The results from the validation study of the dissolution method were all within

the acceptance criteria's, thus allowing application of the method for routine analysis of galantamine hydrobromide tablets [15-16].

5. CONCLUSION

High-Performance Liquid Chromatography (HPLC) for assay and related substances was developed and validated. There was no interference of placebo or the degradations products at the retention time of the drug peak. The method was thus validated and found to be specific, linear, precise, accurate, and robust. Saturation solubility of Galantamine Hydrobromide was determined in different pH mediums and it was found that Galantamine Hydrobromide has pH-dependent solubility, freely soluble in alkaline pH, and insoluble in acidic pH. Drug excipients compatibility study was performed at the accelerated temperature and humidity to observe any physical change concerning the controlled samples. Based on this study, we conclude that the method and technique used were both correct, due to which the exact method and formulation could be made.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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