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METHOD DEVELOPMENT AND VALIDATION OF GALANTAMINE HBr IN PHARMACEUTICAL CAPSULES DOSAGE FORM BY RP-HPLC

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ABSTRACT

The reverse phase high performance liquid chromatographic RP-HPLC method has been developed to quantify galantamine HBr in raw material and capsule formulations using C18 analytical reverse phase column. Mobile phase consisted of solution A and solution B (75:25v/v) pumped at a flow rate of 1ml/min at 250c column temperature. Run time was about 8min with symmetrical peaks. Galantamine HBr was detected by PDA detector at 230 nm with no interference of excipients. The method was linear over a concentration range of(6-28gμ/ml) (R2-1.000). The LOD and LOQ of galantamine HBr were 0.1 and 0.6μg/ml respectively. The result obtained shows a good agreement with declared contents in pharmaceutical formulations. The proposed method is rapid accurate, economical and selective and it may be used for quantification analysis of galantamine in galantamine HBr capsules because of its sensitivity and reproducibility.

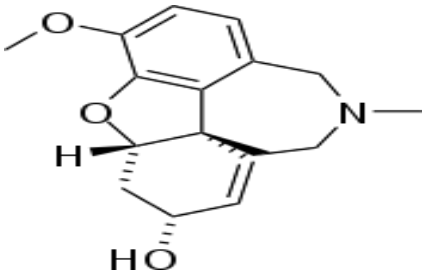
Key Words: RP-HPLC, Galantamine capsules, Galantamine api, Disodium orthophosphate.

INTRODUCTION

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 C17 H21 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.

Systematic* (IUPAC) *name

(4a*S*,6*R*,8a*S*) -5, 6, 9, 10, 11, 12-hexahydro-3-methoxy-11-methyl-4a*H*- [1] benzofuro [3a,3,2-*ef*] [2] benzazepin- 6-ol



There is no any official method for determination of Galantamine Hydrobromide. Different analytical methods like, HPLC (Anonymous 1), micellar electrokinetic chromatography–electrospray ionization mass spectrometry (Anonymous 2), high-performance liquid chromatographic method with UV photodiode-array, fluorescence and mass spectrometric detection (Anonymous 3), RP-HPLC (Anonymous 4) and liquid chromatographic–tandem mass spectrometric method (Paul

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WC *et al.,* 1995) 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.

MATERIALS AND METHODS

HPLC System: Shimadzu autosampler equipped with gradient pump. PDA detector and Intersil ODS-3V column and programmable auto sampler controlled by LC-SOLUTION software.

Chromatographic Parameters

Column: Intersil ODS-3V (250mm*4.6mm), 5µm.
Detector: UV-Visible.
Wavelength: 230nm
Flow Rate: 1ml/min
Mobile Phase: Deggased mixture of mobile phase A (Disodium orthophosphate dehydrate 6.5 buffer: Methanol) and mobile phase B (Deggased mixture of Acetonitrile: Methanol, 95; 5v/v). Mobile phase A: Mobile phase B 75:25 v/v).
Diluent: Mixture of Methanol and Water 95:5 v/v.

REAGENTS

Galantamine HBr API, Galantamine 24mg capsules, Methanol, Acetonitrile, Disodium hydrogen orthophosphate dihydrate- ARgrade, Orthophosphoric Acid.

STANDARD SOLUTION

Preparation of standard stock solution

Standard galantamine HBr 24mg was accurately weighed and transferred to 100ml volumetric flask. About 30ml diluent was added and sonicated until dissolved and dilute up to volume with diluent and mix well (concentration 240µg/ml of galantamine). Now 5ml of this solution was further diluted to get final concentration of 24µg/ml of galantamine HBr.

Preparation of sample solution

The contents of 20 capsules fill weight taken and average fill weight of pellets noted. Make it to fine powder and mix. This sample is only used throughout the validation process. Accurately weigh equivalent amount of fine powder of about 24mg of galantamine sample and transfer into 100 ml volumetric flask. Add 30 ml of diluent and sonicate for 20 minutes with occasional shaking. Equilibrate to room temperature and then dilute up to volume with diluents and shake well. Filter about 10 ml of sample through 0.45µm Nylon filter (concentration 240µg/ml of galantamine HBr). 5ml of this solution transferred to 50ml volumetric flask. Dilute to volume with diluents mix well and fill in auto sampler vial (Varaprasad A *et al*., 2012; Lalitha KG and Venkatachalam T, 2012).

RESULTS AND DISCUSSIONS

The detection wavelength 230 nm was chosen in order to acheive a good sensitivity for quantitative determination of galantamine HBr in capsule dosage form. The mobile phase consisting of solution A : solution B (75:25v/v) in gradient mode offered a good seperation at 25^0C temperature under these conditions using flow rate of 1ml/min and runtime of 8minutes shown in chromatograms, Fig 1(a),(b),(c),(d),(e) which illustrates the seperation of actie ingradients in this system. The gradient program throughout HPLC method was used to analyse galantamine Hydrobromide with greater accuracy. The proposed method is simple and do not involve laborious time consumeing in sample preparation.

Linearity and calibratio curve

The plot peak area response against concentration is shown in fig 1. The plot is linear over a concentration range of 5-35µg/ml of galantamine HBr. Linearity of calibration curve was determined by weighed 1/c least squre reggresion analysis. The co-rrelation coefficient was found to be 1.000 . A linear relationship was found for all components.

System suitability

System suitability and system performance were daily performed during entire validation process of this method. Results are as follows-

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 were tabulated in Table 3.

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 presented in Table 4.

Method Robustness

Robustness of the method was determined by small changes in flow rate,pH,buffer phase composition. The content of drug was not adverselyaffected by these changes as evident from low value of relative standard deviation indicating that method was robust. The results of robustness were presented in Table 5.

Method Ruggedness

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

Specificity

There was no interference from sample, placebo and peak purity of Galantamine Hydrobromide and impurity A were found to be 1.000 and 0.9992 respectively. It showed that developed analytical method was specific for analysis of Galantamine Hydrobromide in capsule dosage form.

Standard and Sample solution stability

Standard and sample solution was evaluated at room temperature for 24 hours. The standard deviation was found to be 2.0%. It showed that both standard and sample was stable up to 24 hours at room temperature.

Table 1. Charecteristics of analytical method derived from standard calibration curve

Compound	Linearity range µg/ml	Linearity equation	Correlation coefficient	Slope of curve
Galantamine HBr	5-35	Y=31026x+1551	1.000	3102.5

Table 2. System suitability and system precision

Compound	RT	n	R	T	%RSD
Galantamine HBr	3.91	7399	0	1.2	0.1
N-oxide	Not detected	Not available	Not available	Not available	Not available
Impurity A	8.26	6832	1.42	1.2	0.1

T=Tailing factor; RT=Retention time; N=Theoritical plates; R=Resolution

Table 3. Method precision

Coumpound	Concentration µg/ml	RT mean	% Assay(n=6)	% RSD of assay(n=6)
Galantamine HBr	24	8.86	100.5	0.2
N-Oxide	24	5.53	100.3	0.2
Impurity A	24	16.65	100.4	0.2

Table 4. Method accuracy

Compound	Level	Drug added(mg)	Drug recovered(mg/ml)	%Assay n=3	%RSD of Assay
Galantamine HBr	50%	0.0121	0.0120	99.7	0.5
	100%	0.0243	0.0242	99.8	0.0
	120%	0.0285	0.0282	99.4	0.4

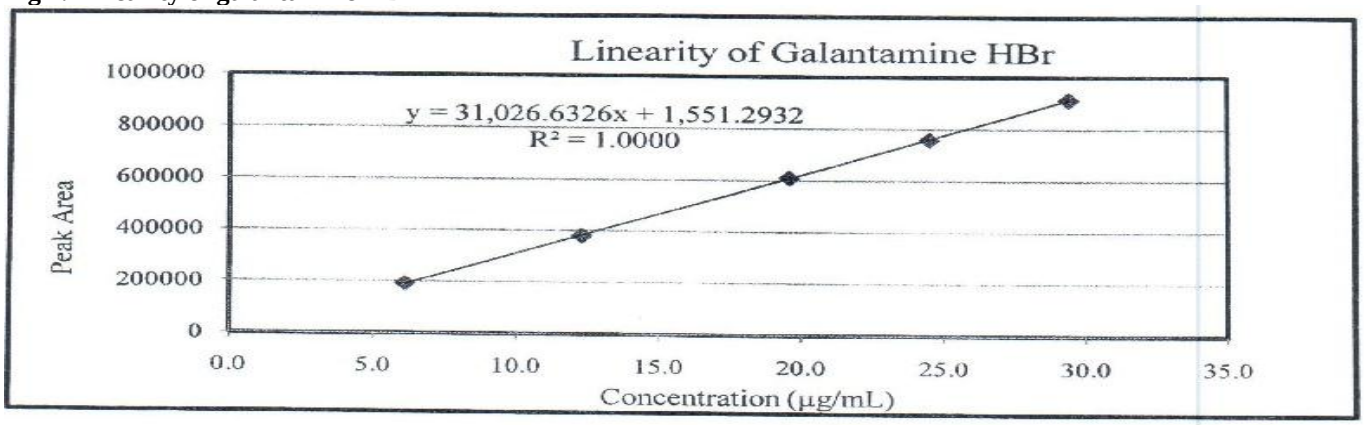
Table 5. Method Robustness(%RSD)in normal and changed condition(n=5)

Compound	Condition	Chage 1ml/min	% RSD
Galantamine HBr	Flow Rate	Normal	
		0.9	0.1
		1.1	0.4
	pH	Normal	
		-0.2 units	0.1
		+0.2units	0.1
	Buffer Phase Composition	Normal	
		73% buffer: 27% Acetonirile	0.1
		77% buffer: 23% Acetonitrile	0.0

Table 6.**Method ruggedness**

Condition	% Assay (n=6)	% RSD of Assay (n=6)
Day 1	Analyst 1,Instrument 1,Column 1 100.7%	0.2
Day 2	Analyst 2,Instrument 2,Column 2 100.7%	0.2

Fig 1. Linearity of galantamine HBr



Linearity Results of Galantamine HBr

S.No	Conc (µg/ml)	Area
1	6.1	191535
2	12.3	381677
3	19.6	611311
4	24.5	760240
5	29.4	914314

Fig 1(a). Chromatogram of Galantamine HBr API

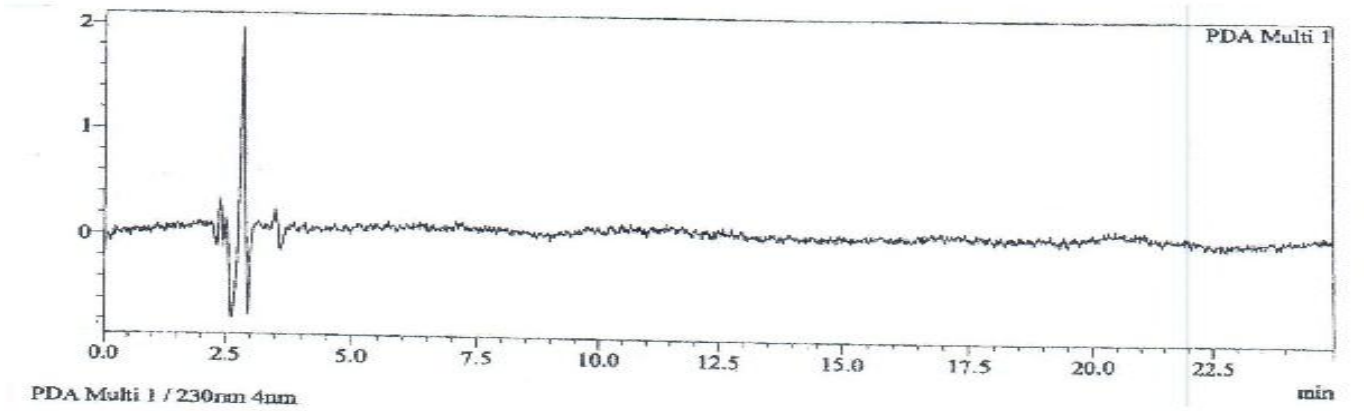


Fig 1(b). Chromatogram of Diluent (Buffer: Acetonitrile)

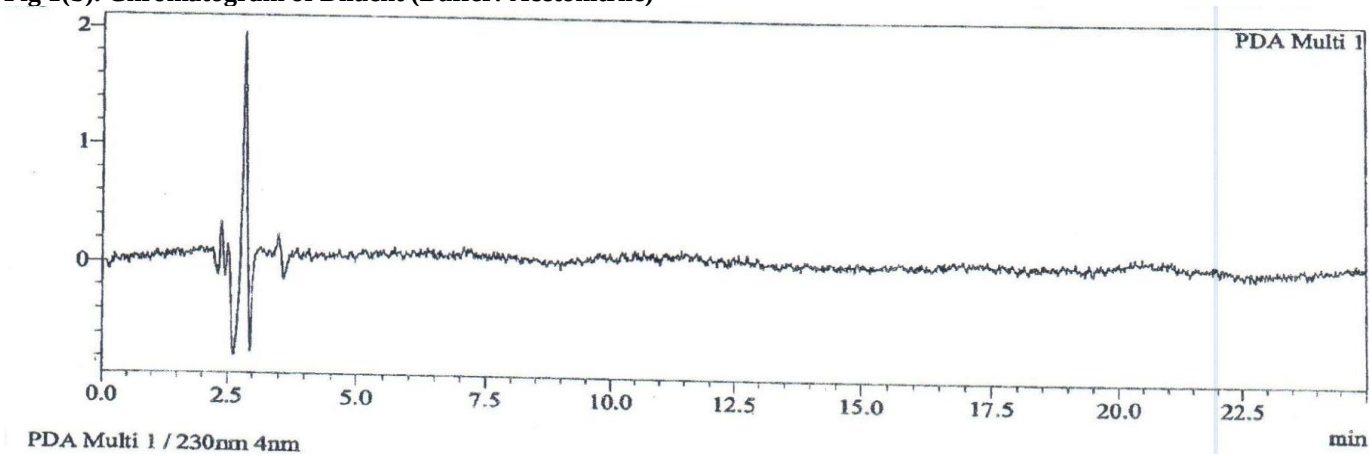


Fig 1(c). Chromatogram of Galantamine HBr Capsule sample solution
Fig(d). chromatogram of Placebo Galantamine HBr
Fig(e). chromatogram of Galantamine HBr Degradant-N-Oxide

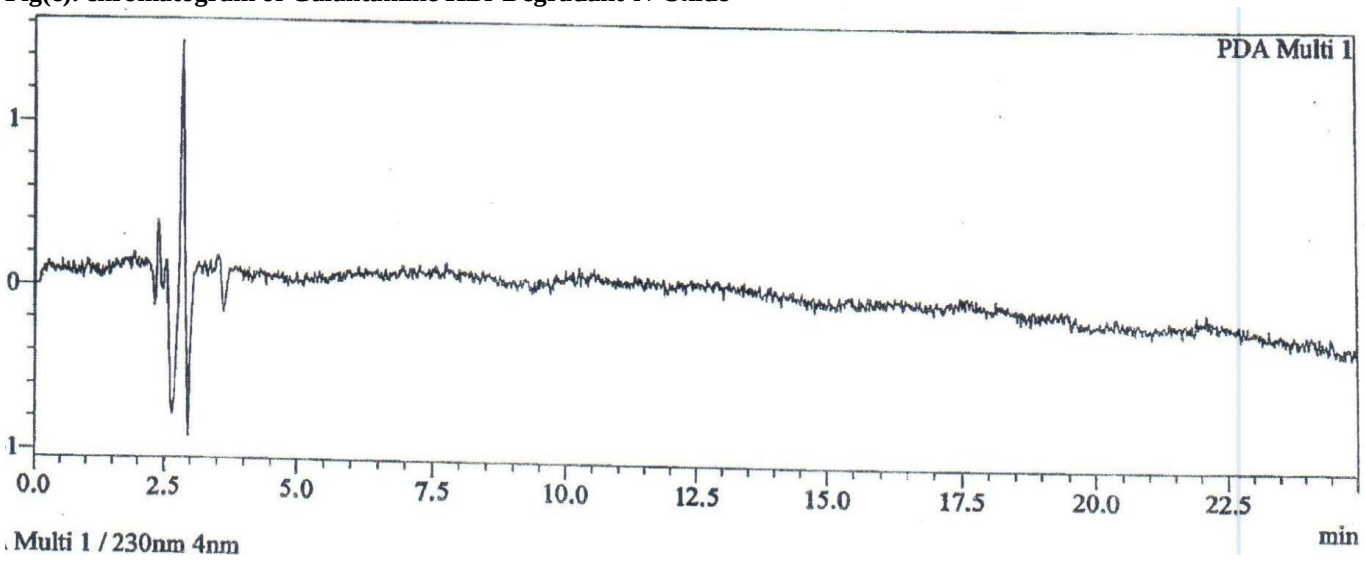
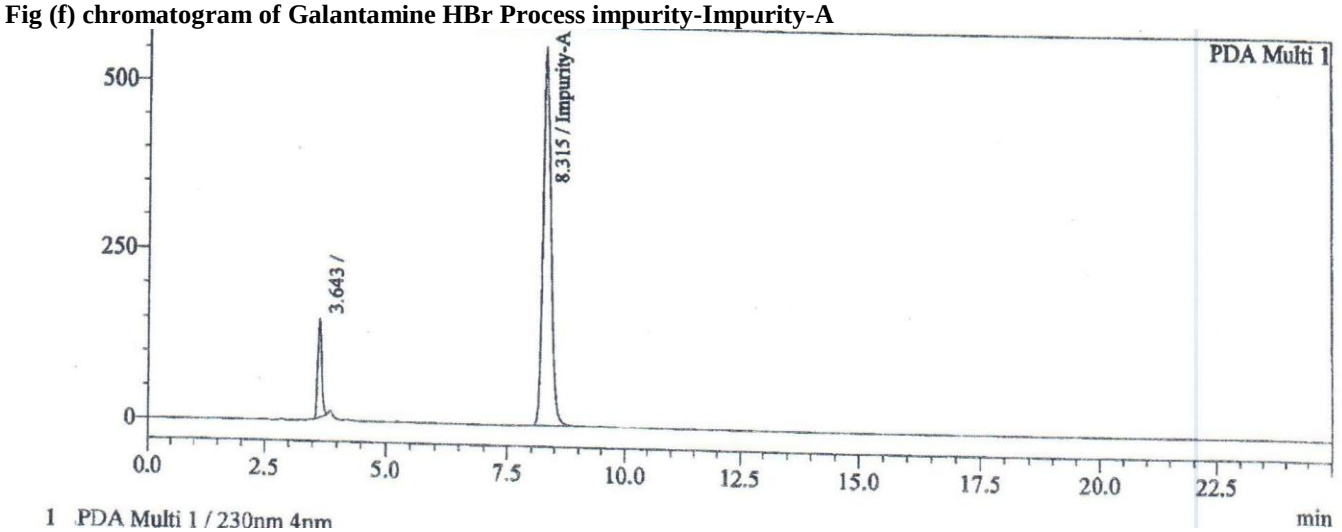


Fig (f) chromatogram of Galantamine HBr Process impurity-Impurity-A



Limits

Limits for system suitability;
%RSD of retention times of the peaks of all the 5 injections is NMT 2%;
% RSD of peak area NMT 2%;
No. of theoretical plates NLT 3000;
Tailing factor NMT 2;
Limits for repeatability and intermediate precision:-
Individual assay must be within 98% to 102%;
% RSD of assay NMT 2%;

CONCLUSION

The method described enables to the quantification of Galantamine Hydrobromide. The advantages lie in the simplicity of sample preparation and low cost of reagents used. The proposed analytical method using HPLC ensure proper resolution and 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 of Galantamine HBr capsules.

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