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Analytical Method Development and Validation of Galantamine HBr ER Capsule in Presence of its Related Impurities by RP-HPLC

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A B S T R A C T

This work was aimed to develop and validate a robust analytical method for detection and quantification of related substances or impurities in galantamine hydrobromide. Galantamine hydrobromide is a key therapeutic agent used in treatment of neurodegenerative disorders such as amnesia. This HPLC (high-performance liquid chromatography) practice was developed using Octadecylsilane carbon column as stationary phase. A gradient elution of buffer and acetonitrile (ACN) in the ratio 97:3 v//v solution used as mobile phase A and 25:75 v/v measured solution and mobile phase B. The detection of λ max was observed at 230 nm and the separation of the component was achieved at a constant temperature of 35°C. Resolution, sensitivity and analysis time was optimized for the method. The results of the developed method demonstrated good resolution, excellent separation of galantamine from its impurities and peak symmetry. By analyzing the reference standard of known impurities as peak purity was within acceptance criteria, the specificity of the method was confirmed. The linearity of the method was demonstrated at concentrations from 8 µg/ml, and the correlation coefficient was shown to be 0.999. The accuracy and precision of this method was confirmed, with the results falling within acceptable limits. Parameters were set for the limit of detection and the limit of quantification for individual corrections. Every aspect of validation including Specificity, Linearity, Accuracy, Precision, Limit of detection, Limit of quantification and System suitability of the method was well received according to the ICH. The developed method is reliable and convenient for the daily analysis of galantamine impurities based on the developed method validation reports. The developed HPLC method allows a sensitive, selective and validated approach for analysis of impurities in galantamine. This method is applicable for galantamine containing pharmaceutical formulations for routine quality control and stability studies therefore, ensuring safety and efficacy of the final product.

Keywords: Analytical method, Impurities, HPLC, Galantamine Hydrobromide, Method development, Validation, Detection.

1. Introduction

Galantamine hydrobromide acts both as reversible competitive acetylcholinesterase inhibitor and Para sympathomimetic in action. Galantamine Hydrobromide is primarily extracted from the source of plants. It is a tertiary alkaloid obtained in nature. Now it has been artificially synthesized in the chemical laboratories. The drug Galantamine hydrobromide is a generic drug that is mainly administered to the person suffering from the loss of memory and forgetful termed Alzheimer's. This drug can be used in both long-term and short-term treatment of the patients has it doesn't completely eliminate the diseases but avoids to get any deeper. The chemical name of the drug was 4as,6r,8as)-5,6,9,10,11,12-hexahydro-3-methoxy-11-methyl-4aH-9[1]benzofuro[3a,3,2-ef][2]benzazepine-6-ol. The drug in the market is distributed in the trade name of Razadyne. The molecular weight of the drug was 287.35. The starting recommended dose 4mg, administered daily twice and the given dose is doubled i.e., 8mg/day after four weeks continued up to the dose reaches to 16-24mg /day in to divided dosage forms i.e., administrating two times per day for an effective pharmacological action of the drug. The starting recommended dose 4mg, administered daily twice and the given dose is doubled i.e., 8mg/day after four weeks continued up to the dose reaches to 16-24mg /day in to divided dosage forms i.e., administrating two times per day for an effective pharmacological action of the drug.

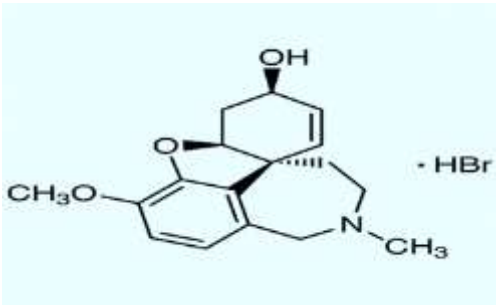


Figure.1 Structure of Galantamine HBr

2. Material and Methodology:

Instruments utilized: The method was developed and validated by usage of semi-automated Alliance model High performance liquid chromatography of Shimadzu with PDA detector and handling over was facilitated by a software version 3 Lab solution. Analytical balance of Sartorius of version SECURA200-10IN, data handling system (Autochrome-3000), Ultra sonicator (UV sonicator) and pH meter (ADWA). The utilized was YMC Pack pro with 150 length,4.6mm diameter and 3µm particle size.

Chemicals: Dibasic potassium phosphate (AR grade, Emparta), Monobasic potassium dihydrogen orthophosphate (AR grade, Emparta), Acetonitrile, Methanol, Isopropyl Alcohol, N-Desmethyl galantamine, Galantamine N-Oxide, Dihydro galantamine, 6S-Galantamine, Anhydro galantamine all these impurities are from Hemarsh. Galantamine HBr (hydrogen bromide) API was from Aurobindo Pvt Ltd.

Selection of UV wavelength: Before starting liquid chromatographic analysis UV scan of the drugs were taken with the help of shimadzu model UV - 1800.The spectrum of the drug shows its best absorbance at wavelength 230nm.

1. Preparation of Buffer: Accurately weigh and transfer about 1.7 gms of Dibasic potassium ortho phosphate (K_2HPO4) and 3.0gms of monobasic potassium phosphate (KH2PO4) into a beaker containing 1000mL of water and mix. Observe the buffer pH at 7.4. The buffer is filtered by using Vacuum filtration through 0.45 membrane filter.

2. Preparation of Mobile phase:

a) Mobile phase A: Solution is prepared by vacuum filtering and gas removed mixture of Buffered solution and ACN in the proportion of 97:3 volume/volume.

b) Mobile phase B: Solution is prepared by vacuum filter and gas removed mixture of Buffered solution and ACN in the proportion of 25:75 volume/volume.

3. Preparation of Diluent: Prepare a filtered and gas removed mixture of Buffered solution & methanol in the ratio of 40:60 volume/volume respectively.

Preparation of solutions:

1. Preparation of Standard Stock Solution: Accurately weighed 25mg of Galantamine working standard is taken into 100ml of vol. flask and fill up to mark by using diluent. Transfer 1ml of above solution into 100ml volumetric flask and fill up by same diluent. This solution contains conc. of 2 µg/ml of galantamine. Vial the solution.

2. Preparation of Sample Solution: Accurately weighed 50mg equivalent wt. of Galantamine HBr (625 mg of crushed sample weight) and taken into 50ml of vol. flask, add 25 mL of diluent & sonicated for 30mins to disintegrate and make up to the mark with the diluent. Filter the preparation through 0.45micron nylon filters. This solution contains 1 mg/ml conc. of galantamine. Vial the solution.

3. Preparation of 100% spiked sample solution: Weigh & transfer 50mg equivalent wt. of Galantamine HBr (625 mg of crushed sample weight) into 50ml of vol. flask, add 25ml of diluent sonicate for 30mins. Spiked specification level of impurities made up to the volume with diluent. Filter the solution through 0.45micron nylon filters. This solution contains 1 mg/ml conc. of galantamine. Vial the solution.

4. Preparation of Placebo Solution: Weigh & transfer 50mg equivalent wt. of Galantamine HBr (525 mg of Placebo powder) into 50ml of vol. flask, add 25ml of diluent sonicate for 30mins to dissolve and make up to mark with the diluent. Filter the solution through 0.45micron nylon filters. This solution contains 1 mg/ml conc. of galantamine. Vial the solution.

Procedure: Separately inject 10µl of diluent, single injection placebo solution, single injection of system suitability solution and sensitivity solution, six replicate injections of diluted. The standard solution and the sample solution are injected into the chromatograph and the chromatogram is recorded, after which the maximum response is measured.

Preparation of System suitability solution: Accurately weigh and transfer 5mg of Galantamine Hydro bromide related impurity mixture RS into a 5mL of volumetric flask, add diluent and sonicate, dissolve dilute to volume with diluent and mix. (1.0mg/m of Galantamine Hydro bromide Related compounds mixture RS).

Suitability requirements:

S/N value should be not less than 10 for Sensitivity solution. The column efficiency as number of theoretical plates for Galantamine Hydro bromide peak NLT 2000. The peak symmetry as Tailing factor for Galantamine Hydro bromide the maximum strength should not exceed 2.0. The relative standard deviation calculated from six replicated injections of Galantamine Hydro bromide peak should be not more than 10.0 %. Resolution not less than 3.4 between galantamine and 6S-galantamine in system suitability solution.

3. Result and discussion:

Optimized Method Development:

Chromatographic system and Parameters

Column	**YMC-Pack Pro, C18,150mm x 3mm, 5micron**
Column Oven Temperature	**35° centigrade**
Detector	**UV & PDA**
λ max	**230nm**
Flow rate	**0.7 mL/min**
Auto Sampler Temperature	**25°C**
Injection volume	**10 ml**
Run time	**35 mins**

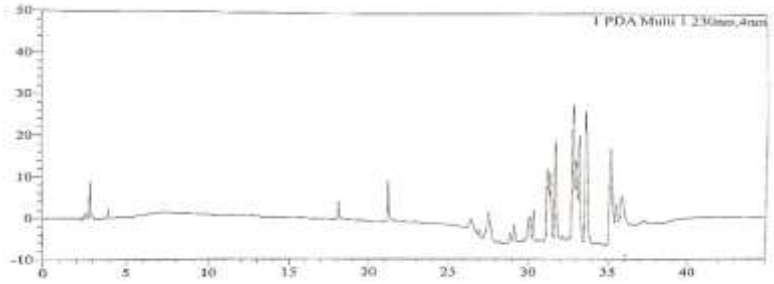


Figure.2 Chromatograph of Blank/Diluent

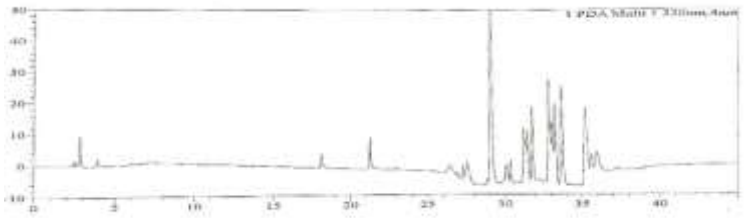


Figure.3 Chromatograph of Placebo Solution

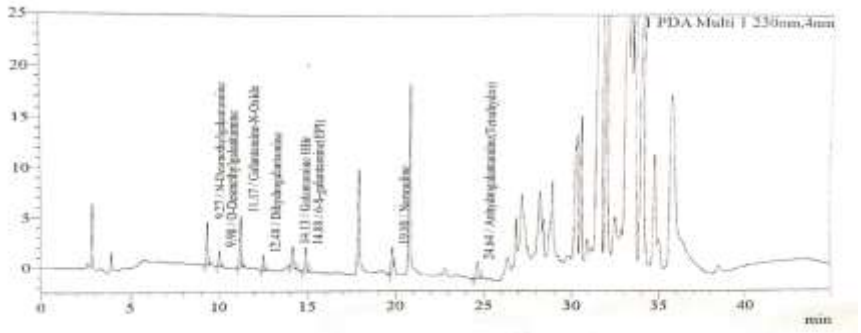


Figure.4 Chromatograph of Spiked Solution of Optimized method

Validation

A. Specificity

Specificity data for Galantamine HBr and its impurities:

Name of the peak	RT (mins)	Area	Maximum peak purity index	RRT
Blank	NA	0	NA	NA
Placebo Interference solution	NA	0	NA	NA
Galantamine HBr of Standard solution	14.70	54740	6154	NA
Galantamine HBr in spiked sample	14.68	20113059	106	1.00
N-Desmethyl galantamine Impurity in spiked sample	9.83	103338	56405	0.67
O-desmethyl galantamine Impurity in spiked sample	10.55	33469	476986	0.72
Galantamine-N-Oxide impurity in spiked sample	11.74	150221	23200	0.80
Dihydro galantamine impurity in spiked sample	13.07	68354	111406	0.89
6-S-Galantamine impurity in spiked sample	15.40	55298	201241	1.05
Narwedine impurity in spiked sample	20.41	91015	136175	1.39
Anhydro galantamine impurity in spiked sample	25.48	85278	157985	1.74

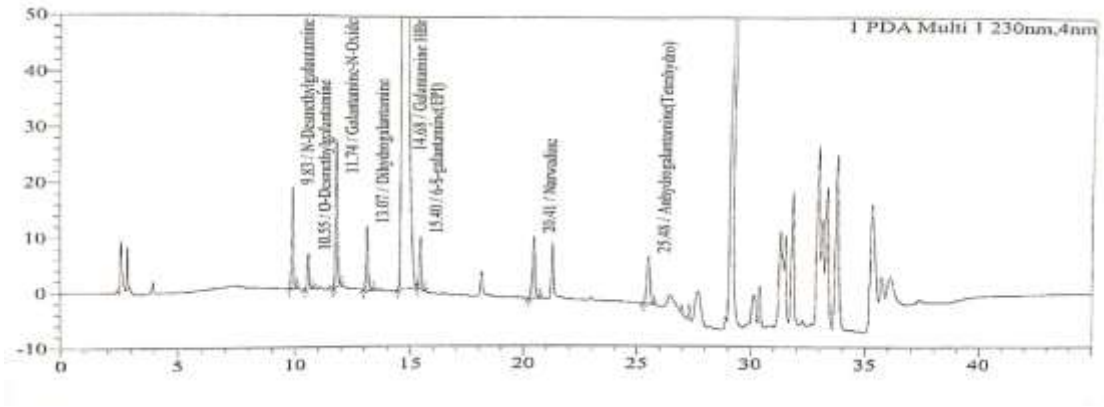


Figure.5 Chromatograph of Galantamine HBr and its impurities

Result: The reports obtained from experiment was observed that the placebo, blank and known impurity peaks were adequately resolved from analyte peak and no interference was observed at the retention time of Galantamine HBr.

B. Linearity:

Table for Systemic suitability and system performance results for Linearity Study:

Parameters	**Result**	**Acceptance criteria**
S/N Ratio for sensitivity solution	60	S/N value should be NLT 10 for sensitivity solution
Theoretical plate count	115177	NLT 2000
Tailing factor	1.1	NMT 2.0
%RSD	0.1	NMT 5.0%
Resolution	5.1	NLT 3.4 between Galantamine & 6-S-Galantamine
System performance	0.1	NMT 5.0%

Linearity data for Galantamine HBr

Level (%)	Concentration (µg/ml)	Peak Area
10	0.1993	7261
25	0.4982	16618
50	0.9964	32059
75	1.4947	47814
100	1.9929	63695
120	2.3915	75697
150	2.9893	92667

Regression data of Galantamine HBr

Slope	30851
Intercept	1421.555
Correlation Coefficient	0.9998
R Square	0.9997
Response	62905.003
Y intercept at 100% level	2.3
Slope of Galantamine	30851
RRF	1.00

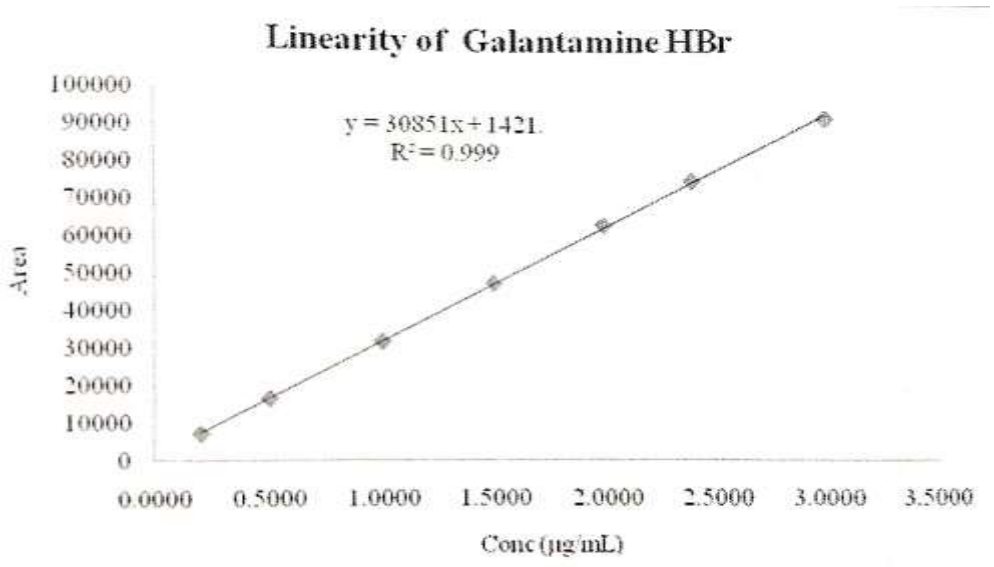


Figure.6 Chromatograph of Linearity of Galantamine HBr

Linearity data for N-Desmethyl galantamine

Level (%)	Concentration (µg/ml)	Peak Area
10	0.5235	10660
25	1.3088	26054
50	2.6176	51930
75	3.9263	77852
100	5.2351	103451
120	6.2821	122736
150	7.8527	153223

Regression data of N-Desmethyl galantamine

Slope	19464
Intercept	837.967
Correlation Coefficient	1.0000
R Square	0.9999
Response	102732.093
Y intercept at 100% level	0.8
Slope of Galantamine	30851
RRF	0.63

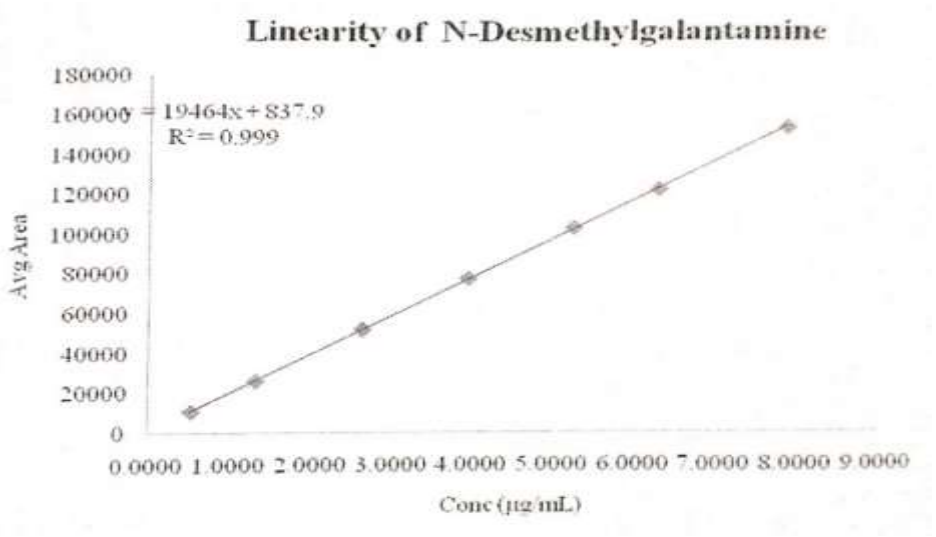


Figure.7 Linearity plot of N-Desmethyl Galantamine Impurity

Linearity data for Galantamine N-Oxide Impurity

Level (%)	**Concentration (µg/ml)**	**Peak Area**
10	0.5259	14016
25	1.3148	34386
50	2.6296	67408
75	3.9445	101448
100	5.2593	134271
120	6.3112	159207
150	7.8889	198396

Regression data of Galantamine N-Oxide Impurity

Slope	25048
Intercept	1563.768
Correlation Coefficient	0.9999
R Square	0.9999
Response	133297.330
Y intercept at 100% level	1.2
Slope of Galantamine	30851
RRF	0.81

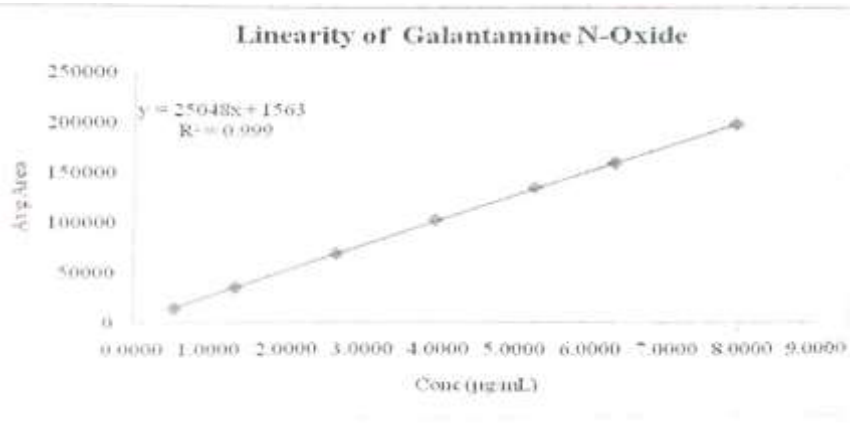


Figure.8: Linearity plot of Galantamine N-oxide Impurity

Result: The reports obtained from experiment was observed that the Analytical Method for the Galantamine HBr ER Capsule has been met the acceptance criteria for linearity of correlation coefficient >0.999.

C. Limit of Detection and Limit of Quantification: The procedure was done for the identification and estimation of quantity of available impurities in the given Galantamine HBr Spiked solution.

Systemic suitability and system performance results or LOD & LOQ Study:

Parameters	**Result**	**Acceptance criteria**
S/N Ratio for sensitivity solution	61	S/N value should be NLT 10 for sensitivity solution
Theoretical plate count	110909	NLT 2000
Tailing factor	1.1	NMT 2.0
%RSD	0.2	NMT 5.0%
Resolution	5.0	NLT 3.4 between Galantamine & 6-S-Galantamine
System performance	0.4	NMT 5.0%

LOD and LOQ data for Galantamine HBr and its impurities with respect to sample concentration.

Component	**Concentration (µg/ml)**		**Concentration(µg/ml)**	
	LOD	LOQ	LOD	LOQ
N-Desmethyl galantamine	0.017	0.05	0.002	0.005
Galantamine N-Oxide	0.017	0.05	0.002	0.005
Dihydro galantamine	0.007	0.02	0.001	0.002
6S-Galantamine	0.007	0.02	0.001	0.002
Anhydro galantamine	0.007	0.02	0.001	0.002
Galantamine HBr	0.007	0.02	0.001	0.002

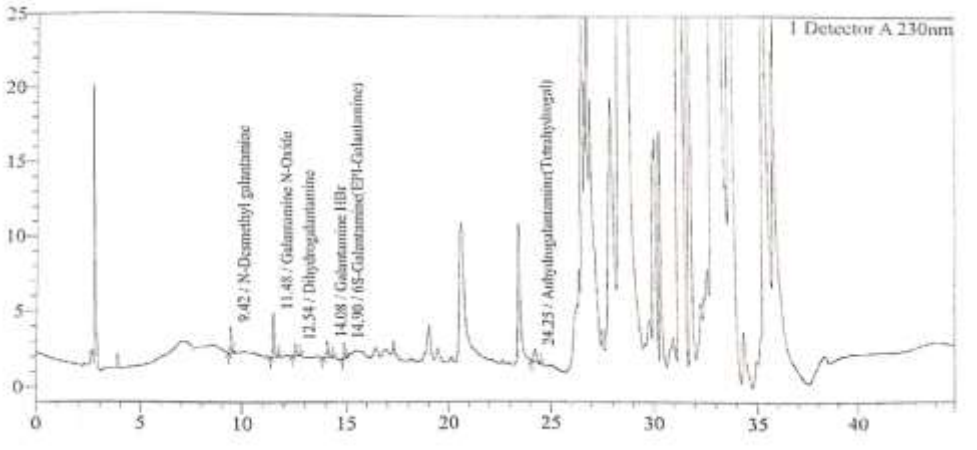


Figure.9 LOQ of Galantamine HBr and its impurities

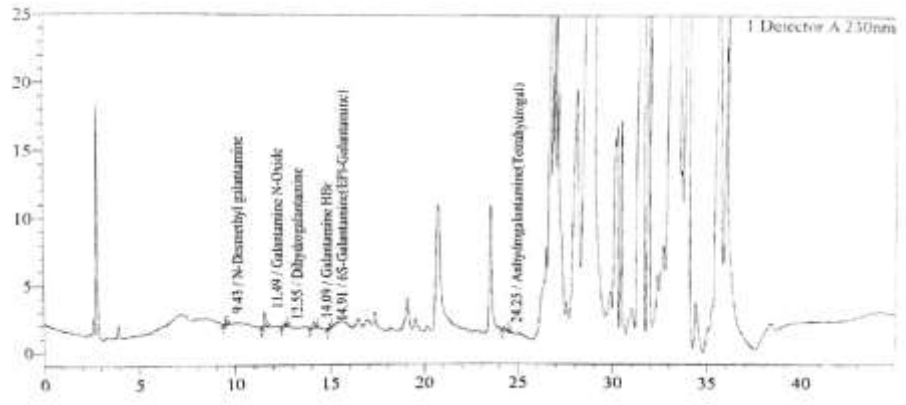


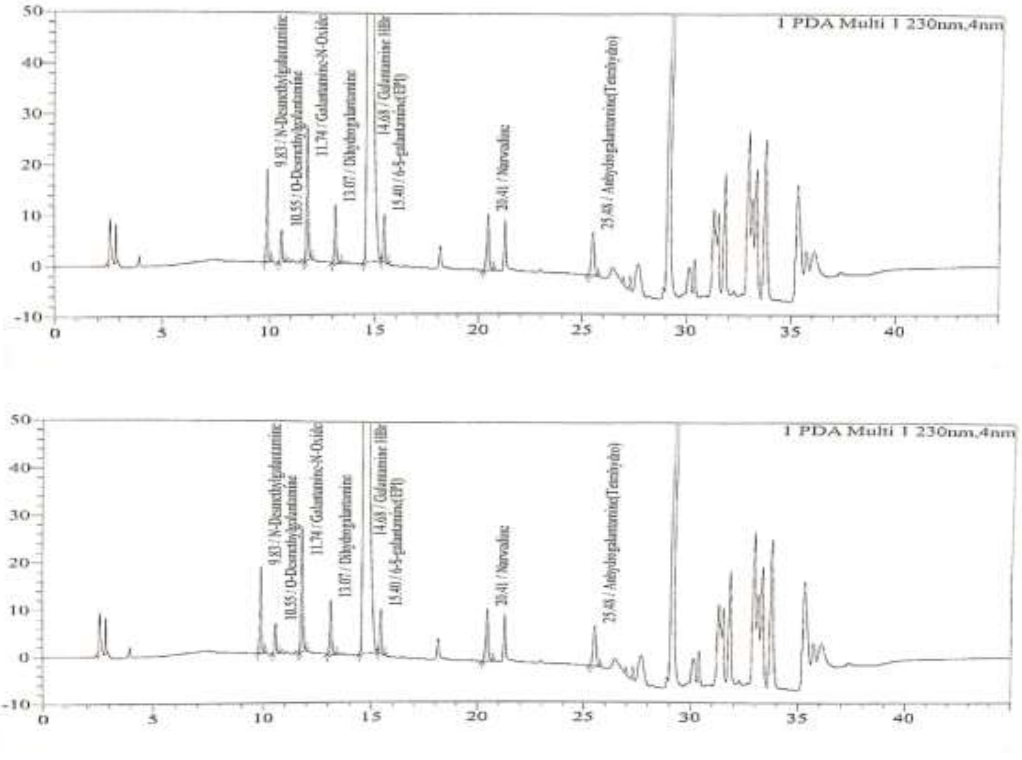
Figure.10 LOD of Galantamine HBr and its impurities

Result: The reports obtained from experiment was observed that the % RSD for precision at LOQ level solution NMT 10.0 and the Analytical method developed for the Related Compounds of Galantamine HBr ER Capsule 8mg has been met the acceptance criteria for LOQ parameter.

D. Method Precision:

Method precision data for the Spiked sample solution

Injection No.	N- Desmethyl galantamine	Galantamine N- Oxide	Dihydro galantamine	6S- Galantamine	Anhydro galantamine
1	105.0	108.4	100.8	103.3	104.1
2	105.0	108.4	100.8	103.3	104.1
3	107.0	108.4	100.8	103.3	104.1
4	105.1	106.5	100.8	103.3	104.1
5	107.0	104.6	100.8	103.3	104.1
6	107.0	106.5	100.8	103.3	104.1
Average	106.0	107.1	100.8	103.3	104.1
%RSD	1.0	1.4	0.0	0.0	0.0



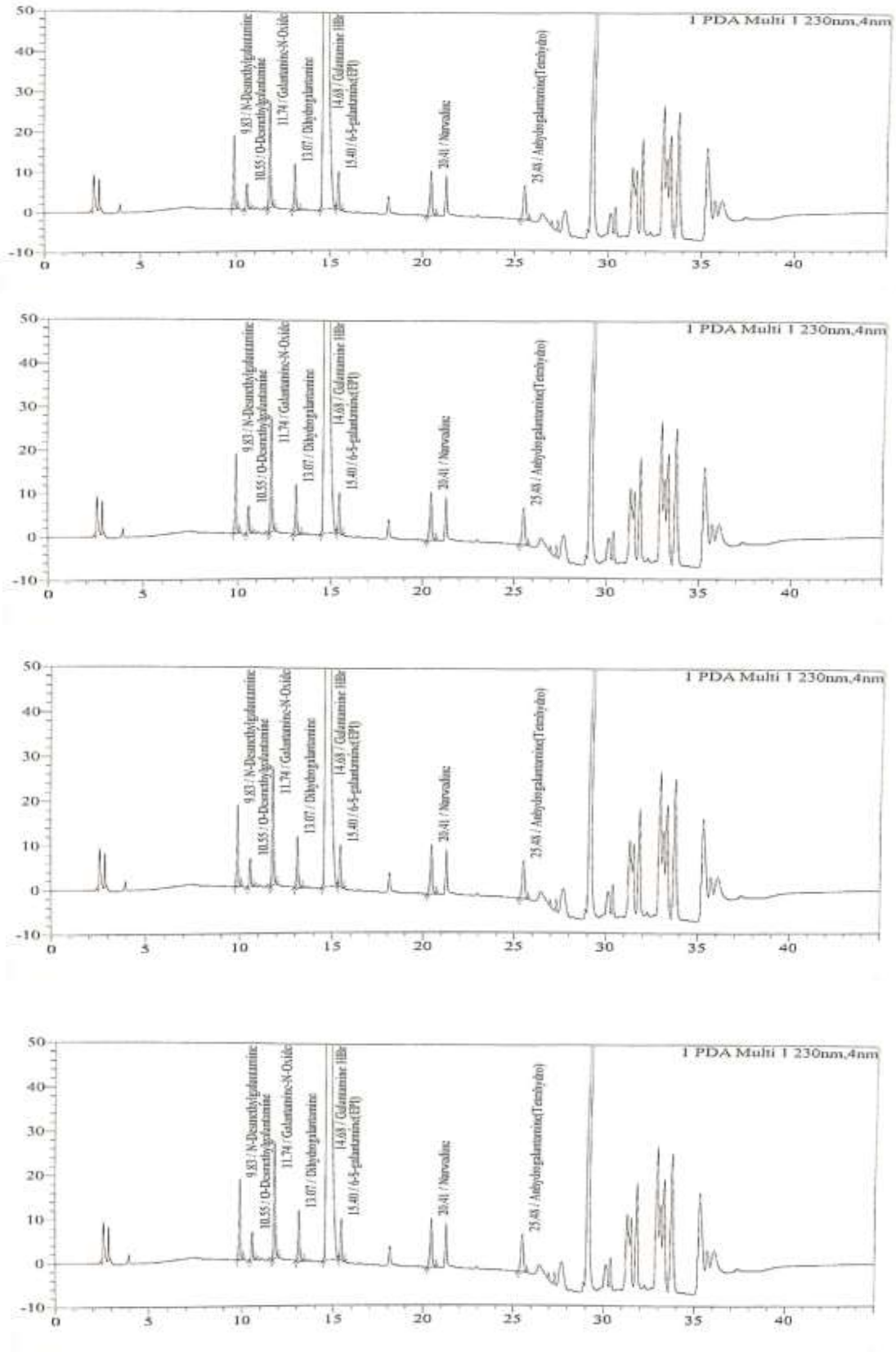


Figure.10 Method Precision of Galantamine HBr impurities

Result: The reports obtained from experiment was observed that the Average Recovery of six formulations at 100% spiking level fall in range of 85% and 115%. The %RSD at 100% level is NMT 10.0% and

E. Solution Stability: The stability of Sample solution is ensured when the difference in percent observed between the impurity values of the stored sample solution, the initial impurity value for peaks > 0.05 should not be more than 20.0%. AS per for the reference solution the system suitability criteria should pass for every time interval. In case of Standard solution, stability is established that percent found value of the stored standard solution, quantitated by the freshly formulated standard solution falls in range of 98.0% and 102.0%.

Table of Stability data for the Plain sample solution

Plain sample						
	Initial	Difference (%)	24 Hours (%)	Difference (%)	48 Hours (%)	Difference (%)
N-Desmethyl galantamine	0.06	NA	0.06	0.0	0.07	16.7
Galantamine N-Oxide	0.11	NA	0.15	36.4	0.15	36.4
Dihydro galantamine	0.18	NA	0.18	0.0	0.18	0.0
6S-Galantamine	0.00	NA	0.01	NA	0.00	NA
Anhydro galantamine	0.08	NA	0.08	0.0	0.08	0.0
RRT	0.06	NA	0.09	50.0	0.08	33.3
Total	0.49	NA	0.57	16.3	0.56	14.3

Table of Stability data for the Spiked sample solution (% Recovery)

Time point	N-Desmethyl Galantamine	Galantamine N-Oxide	Dihydro galantamine	6S-Galantamine	Anhydro galantamine
Initial	105.1	108.4	100.8	103.3	104.1
24th Hour	105.1	106.5	100.8	98.8	99.5
% Diff	0.0	1.9	0.0	4.5	4.6
48th Hour	112.7	119.8	105.9	94.3	108.6
% Diff	7.6	11.4	5.1	9.0	4.5

Result: The reports obtained from experiment was observed that the Standard solution is stable up to 24 hours when stored at room temperature. Spiked Sample solution is stable up to 24th hour when stored at room temperature. Plain sample is not stable up to 24th hour. Inject the freshly prepared samples.

4. Conclusion:

Galantamine hydrobromide acts both as reversible competitive acetylcholinesterase inhibitor and Parasympathomimetic in action. Survey on this drug product exposed there are less methods developed analytically for the Galantamine Hydrobromide by UV and HPLC in laboratories. By using universal RP-HPLC a unique method was developed or Galantamine which sensitive in detection, accurate, reproducible and is convenient for use where it is always a need of reporting various products less period of time. Hence, this RP-HPLC method was developed using C18 column as stationary phase. A gradient elution of buffer and acetonitrile (ACN) in the ratio 97:3 (volume/volume) and 25:75 (volume/volume) used as mobile phase A and mobile phase B respectively. The detection of λ max was observed at 230 nm and the separation of the component was achieved at a constant temperature of 35℃. Linearity of the method was over a concentration range of 8 µg/ml where correlation coefficient was observed 0.999. Limit of quantification, Limit of detection, specificity, accuracy is in accordance with the specified limits of the USP guidelines. The developed method is validated and results shows that method developed is suitable for the intended purpose.

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