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

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Abstract: A detailed review of literature showed that different methods have been developed for detecting Galantamine HBr in pharmaceutical formulations, but these methods are costly and require sophisticated equipment's. So the present study is designed to develop an assay method for the estimation of Galantamine HBr drug by RP-HPLC in the capsule formulation. The method is validated as per ICH guidelines. Solubility data and Analytical data were studied and undertaken to develop initial conditions. Initial chromatographic conditions for assay of Galantamine HBr (24mg) in tablet dosage form were established and optimized. Analytical Method validation was done for the developed HPLC methods as per ICH guidelines.

Keywords: Galantamine HBr, RP-HPLC, Method Development, Chromatographic Conditions, ICH guidelines.

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PAPER-QR CODE

INTRODUCTION

Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. It seeks ever improved means of measuring the chemical composition of natural and artificial materials. This branch of chemistry, which is both theoretical, and a practical science, is practiced in a large number of laboratories in many diverse ways while analytical method, is a specific application of a technique to solve an analytical problem. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. The discipline of analytical chemistry consists of qualitative and quantitative analysis.

Qualitative analysis establishes the chemical identity of the species in the sample. **Quantitative analysis** determines the relative amounts of these species, or analytes, in numerical terms.

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS[3]

Instrumental method is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied sciences. Analytical Instruments plays an important role in the production and evaluation of new products. This instrumentation provides lower detection limits required to assure safe foods, drugs, water and air. Instrumental methods are widely used by Analytical chemists to save time, to avoid chemical separation and to obtain increased accuracy. Most instrumental techniques fit into one of the four principal areas, i.e., spectroscopy, electrochemistry, chromatography and miscellaneous techniques[4,5].

Most instrumental techniques fit into one of the four-principle areas[6-10]

A) Spectrophotometric techniques:

- UV and Visible Spectrophotometry
- Fluorescence and Phosphorescence Spectrophotometry
- Atomic Spectrophotometry (emission &absorption)
- Infrared Spectrophotometry
- Raman Spectrophotometry
- X-Ray Spectrophotometry
- Nuclear Magnetic Resonance Spectroscopy

- Mass Spectroscopy
- Electron Spin Resonance Spectroscopy

B) Electrochemical Techniques

- Potentiometry
- Voltametry
- Electrogravimetry
- Conductometry
- Amperomertry

C) Chromatographic Techniques

- High Performance Liquid Chromatography
- Gas chromatography
- High Performance Thin Layer Chromatography
- Thin Layer Chromatography
- GC- MS (Gas chromatography - Mass Spectroscopy)
- LC-MS (Liquid Chromatography - Mass Spectroscopy)

D) Miscellaneous Techniques

- Thermal analysis
- Kinetic Techniques
- Electrophoresis

Reverse phase chromatography

Reverse phase chromatography - a bonded phase chromatography technique, uses water as base solvent. Separation is based on solvent strength and selectivity. Separation is also affected by column temperature and pH. In general, the more polar compounds elute faster than the less polar compounds. UV detection is the most common detection technique used[11-3].

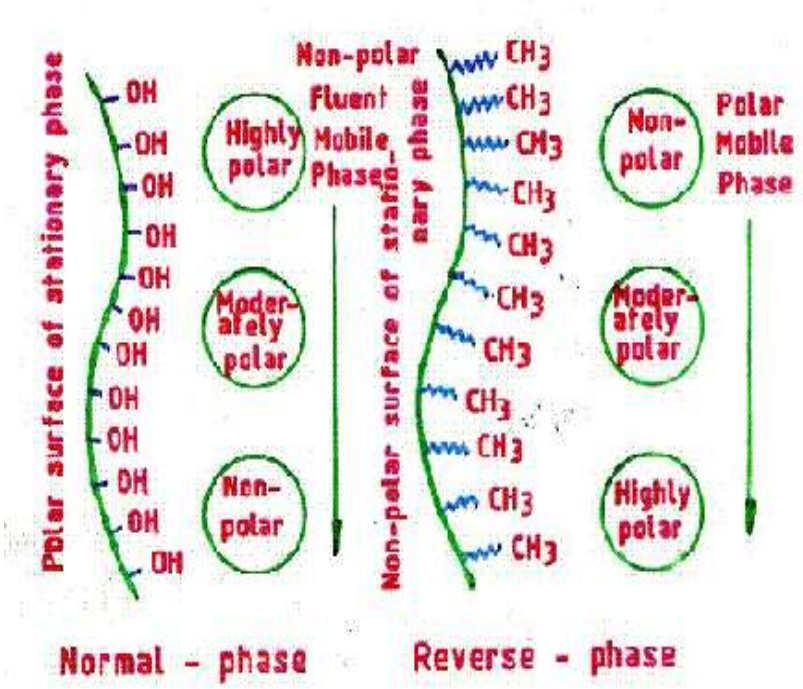


Fig.1.1: Skeleton of Normal and Reverse phases

Certain limitations of RP-HPLC are[14-16]:

- Compounds much more polar than the compound of interest may be masked (eluted together) in the solvent front / void volume.
- Compounds very less polar than the analyte may elute either late during the chromatographic run or are retained in the column.
- The compounds with lower UV extinction coefficients or different wavelengths maxima may not be detectable at the low level relative to the visibility of the analyte since only one or two wavelengths are monitored.

DRUG PROFILE

Galantamine HBr:

Galantamine HBr is a parasympathomimetic .It is a reversible, competitive acetylcholinesterase inhibitor. A benzazepine derived from norbelladine.

Chemical name : (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.

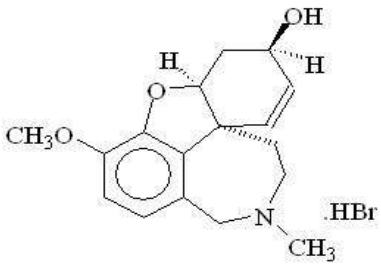
Empirical formula : $C_{17}H_{21}NO_3$. HBr.

Molecular weight : 368.27.

Melting point : 126.5 °C (260 °F)

Solubility : sparingly soluble in water insoluble chloroform, ether and alcohol

Structural Formula



MATERIALS USED

Purified water, Hydro chloric acid, Methanol**,** Acetonitrile**,** Disodium hydrogen orthophosphate**,** Glacial acetic acid, Sodium hydroxide**,** Acetonitrile, Ortho phosphoric acid.

METHOD DEVELOPMENT

VALIDATION DATA OF GALANTAMINE BY RP-HPLC

The following experimental design is drawn in order to prove the test method is capable to yield consistent, reliable and reproducible results within the pre-determined acceptance limits.

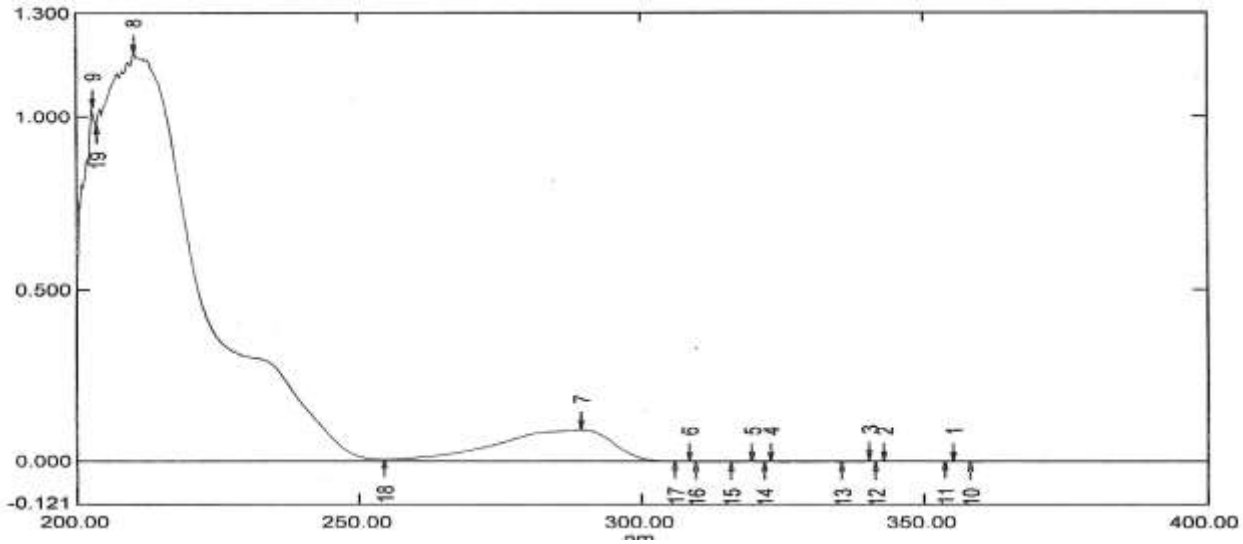
- Acceptance criteria for above validation parameters are specified in individual experimental design.
- Observations and results are recorded in individual method validation data sheets.
- Summarize the findings of the method validation and draw interference.
- Based on the interpretation of the results in method validation, draw the conclusion.

The following parameters have been validated.

1. Linearity
2. Precision

3. Accuracy
4. Specificity
5. Robustness
6. Ruggedness.

EXPERIMENTAL WORK



No.	Wavelength nm.	Absorbance	Description
1	230.00	0.309	

Fig.No.1: UV spectra of Galantamine HBr

Table.No.1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Parameter	Conditions
Stationary phase	Inertsil ODS-3V (length :250mm Internal diameter : 4.6mm Particle size : 5µm)
Mobile phase	**A** : 0.05M disodium hydrogen phosphate dihydrate : Methanol(95:5), **B**: Acetonitrile : Methanol (95:5). Solution A : Solution B – **75:25**

Flow rate(ml/min)	1.0
Column back pressure (kg/cm^2)	80 – 85
Run time (min)	8
Column temperature (^0C)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	By UV at 230 nm
Internal standard	-

Fig.No-2-Optimized Standard Chromatogram

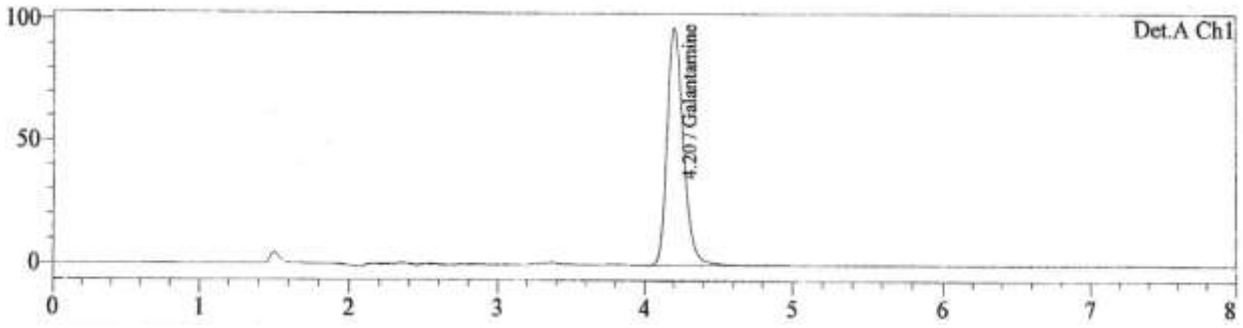
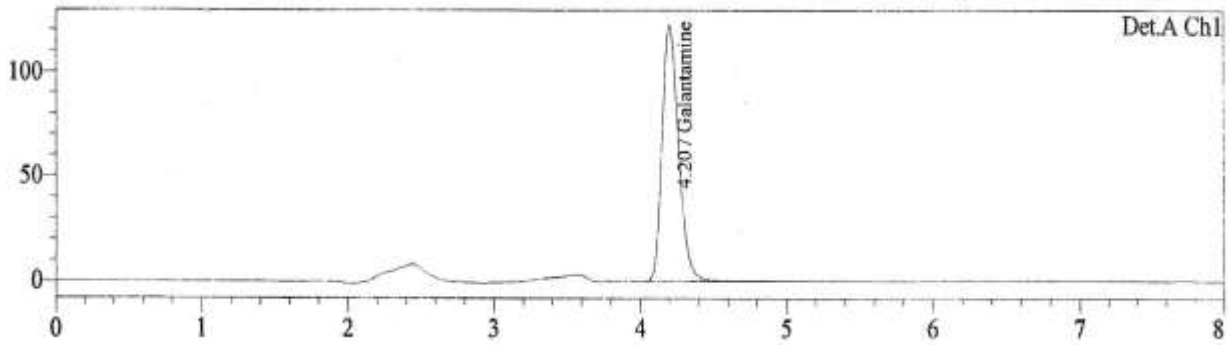


Fig.No-3-Optimized Sample Chromatogram



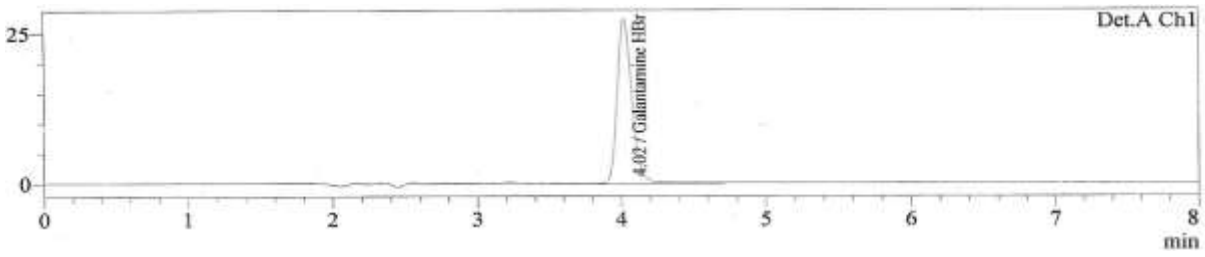


Fig.No-4-Chromatogram showing linearity level-1 (6ppm)

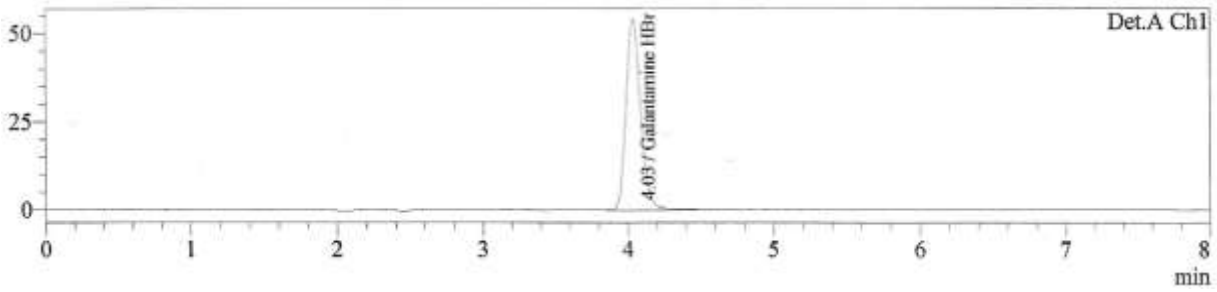


Fig .No.5: Chromatogram showing linearity level-2 (12ppm)

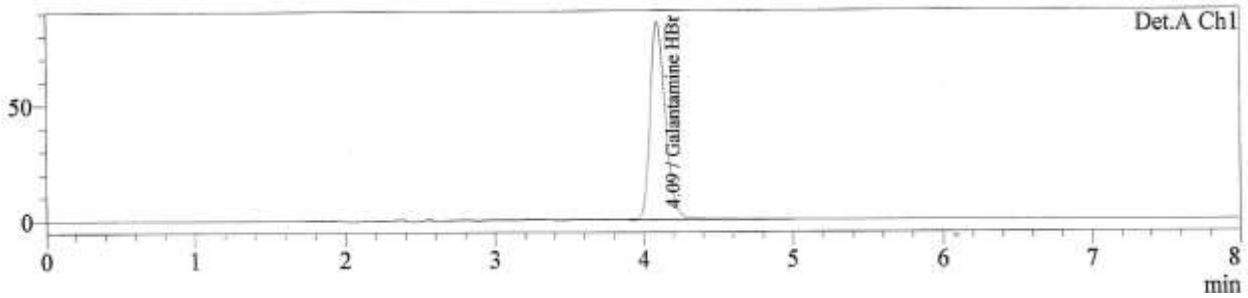


Fig .No.6: Chromatogram showing linearity level-3 (24ppm)

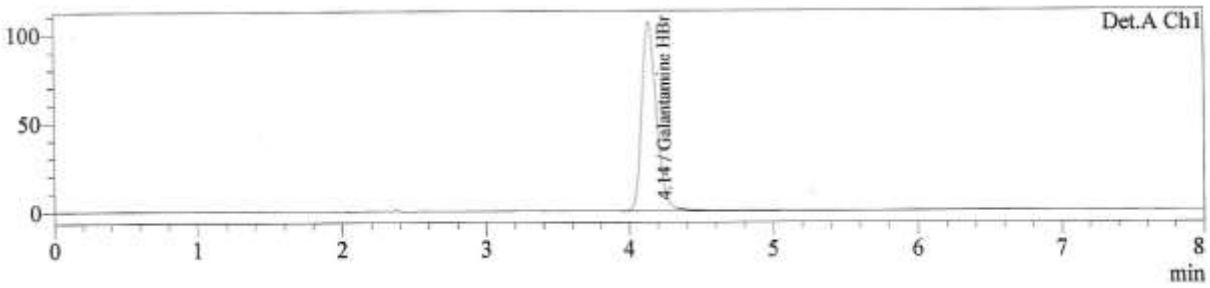
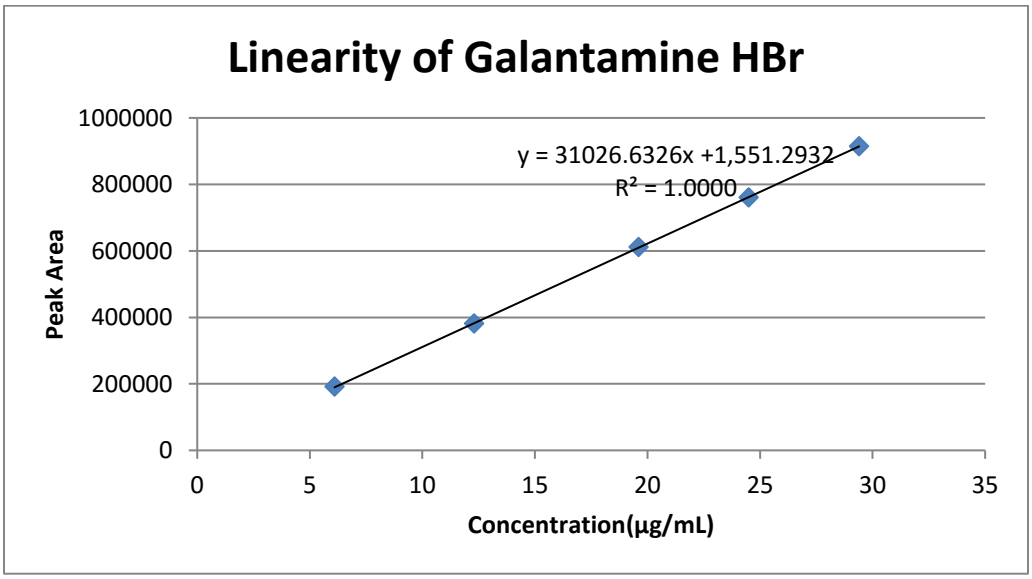


Fig .No.7: Chromatogram showing linearity level-4 (30ppm)

Table.No.2: showing results from linearity study

Actual concentration(%)	w.r.t Specification Level	Concentration (µg/mL)	AREA	AVG AREA	SD	%RSD
25	6.1	6.1	191970	191535	414.70	0.2
			191144			
			191492			
50	12.3	12.3	381667	381677	387.59	0.1
			382069			
			381294			
80	19.6	19.6	610741	611311	751.96	0.1
			611028			
			612163			
100	24.5	24.5	760529	760240	600.51	0.1
			760642			
			759550			
120	29.4	29.4	914422	914341	772.69	0.1
			915070			
			913531			

Fig .No.8: Plotting of calibration curve for Galantamine HBr



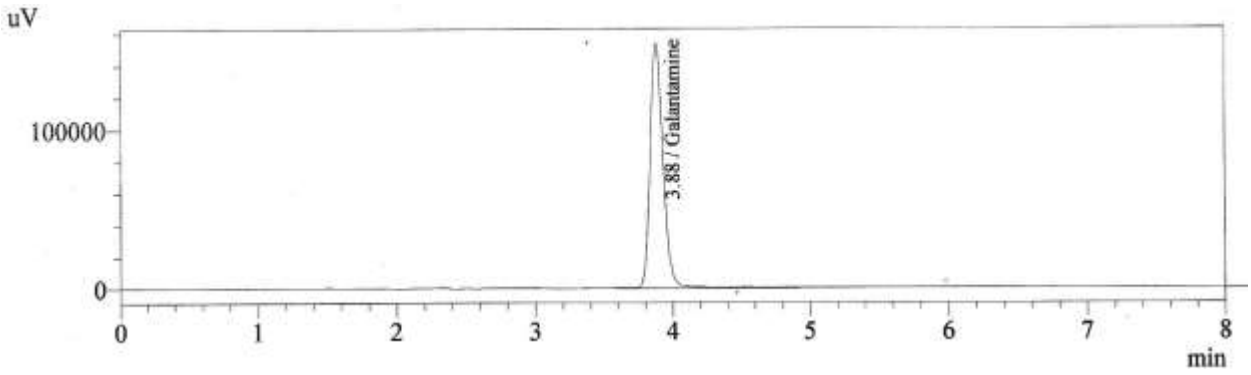


Fig .No.9: Chromatogram showing precision injection-1

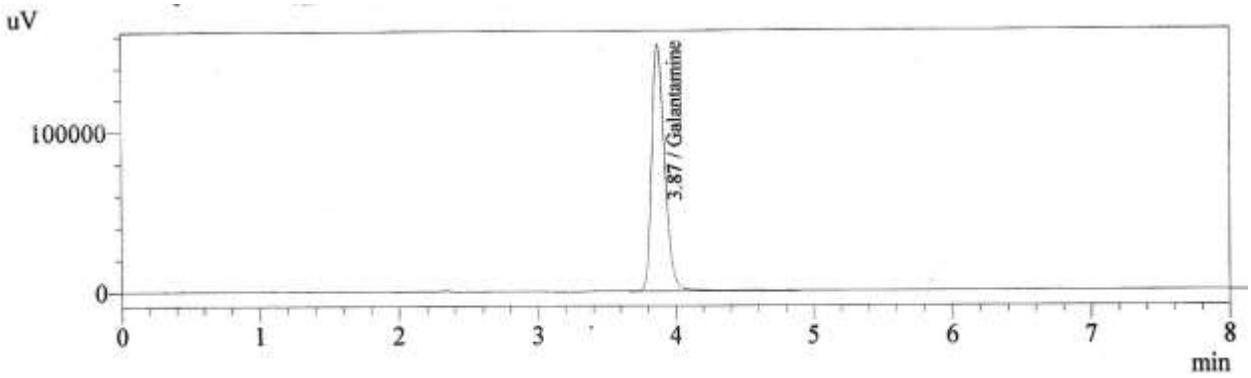


Fig .No.10: Chromatogram showing precision injection-2

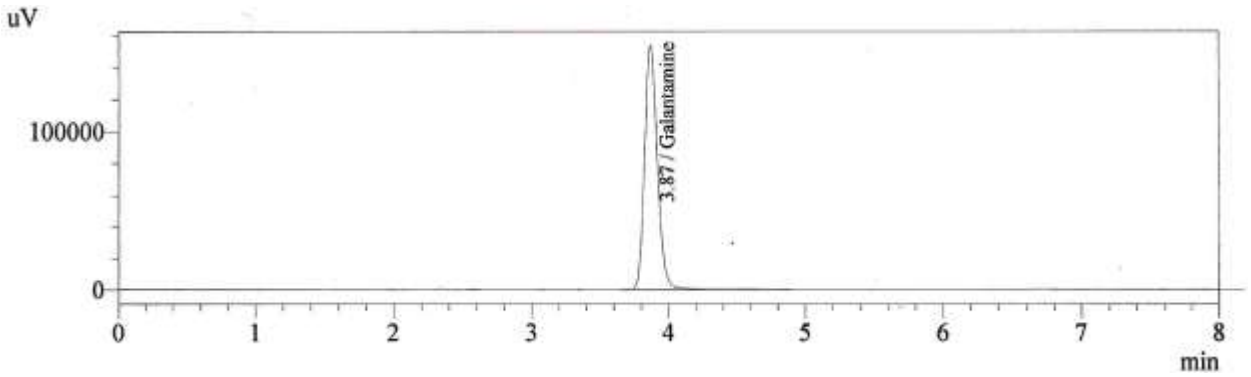


Fig .No.11: Chromatogram showing precision injection-3

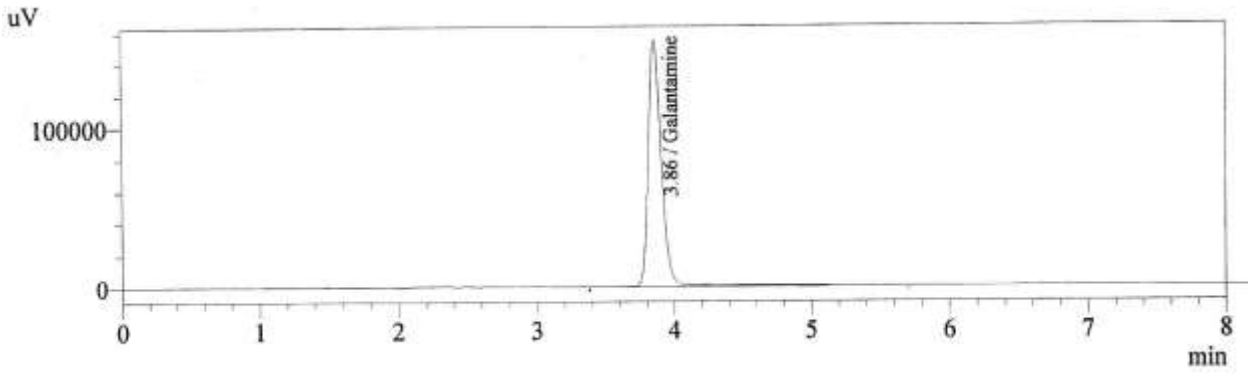


Fig .No.12: Chromatogram showing precision injection-4

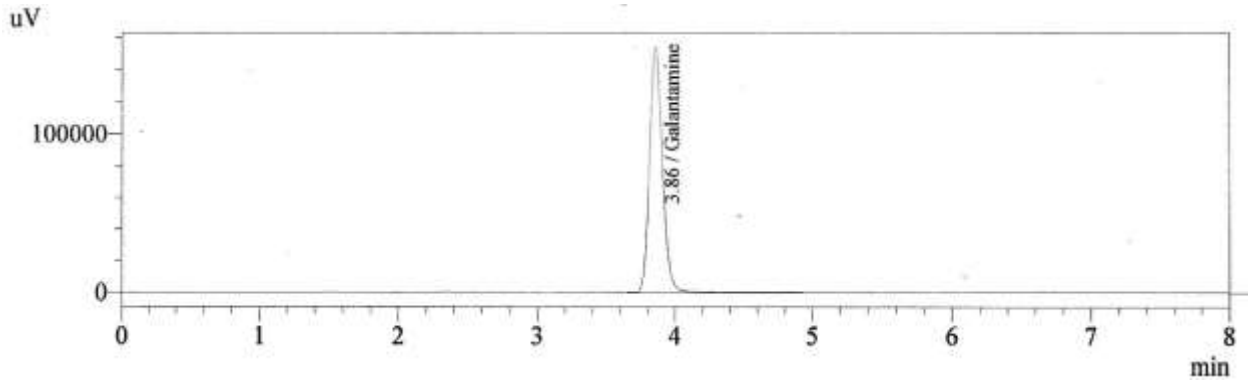


Fig .No.13:Chromatogram showing precision injection-5

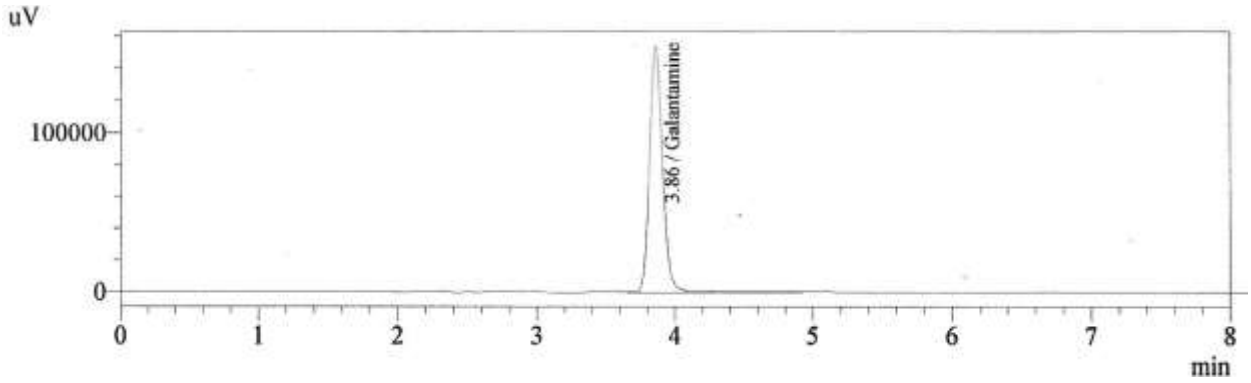


Fig .No.14: Chromatogram showing precision injection-6

Table.No.3: Precision of galantamine HBr

Sample	Area	Sample weight	% Assay
1	1003919	302.12	100.8
2	1002431	301.72	100.8
3	1002307	301.92	100.7
4	1003782	302.12	100.8
5	1001057	302.12	100.5
6	1002724	303.12	100.3

Mean	100.7
SD	0.21
%RSD	0.2

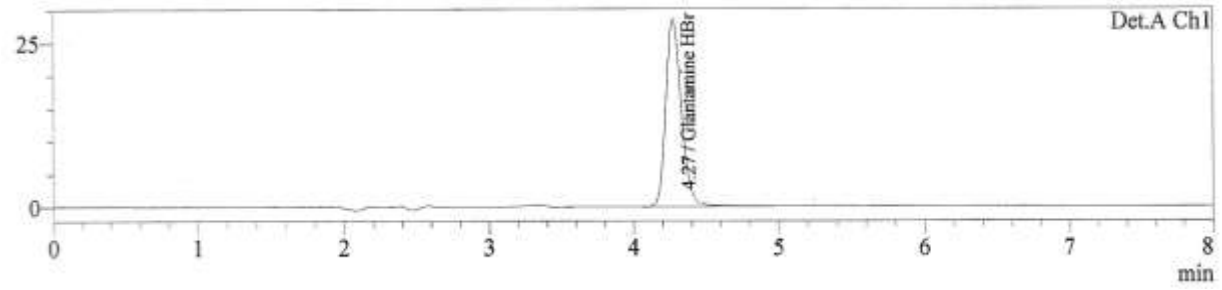


Fig .No.15: Chromatogram showing accuracy-25% injection

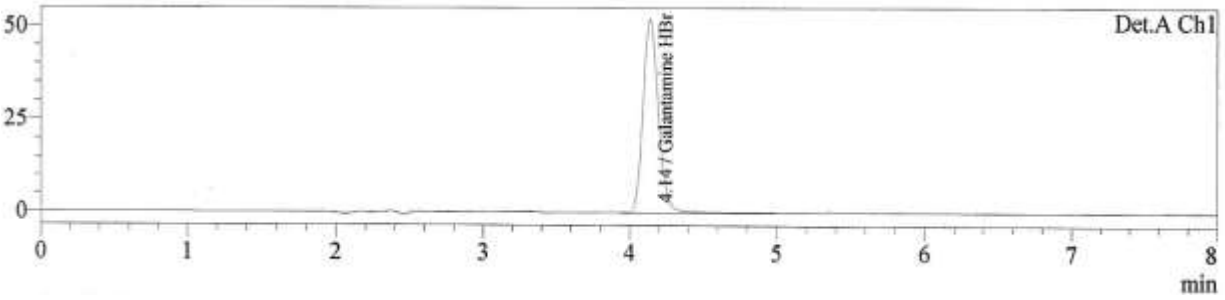


Fig .No.16: Chromatogram showing accuracy-50% injection

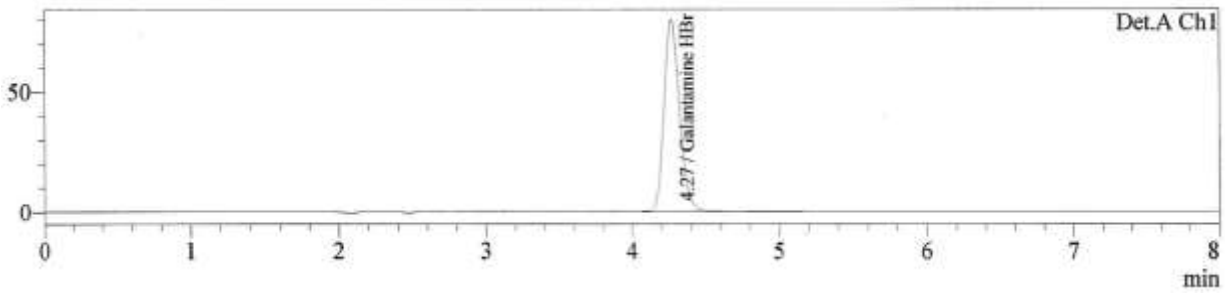


Fig .No.17: Chromatogram showing accuracy-80% injection

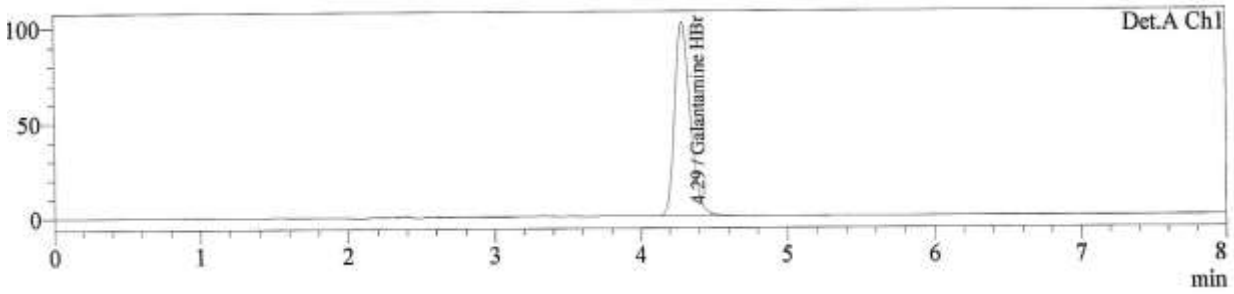


Fig .No.18:Chromatogram showing accuracy-100% injection

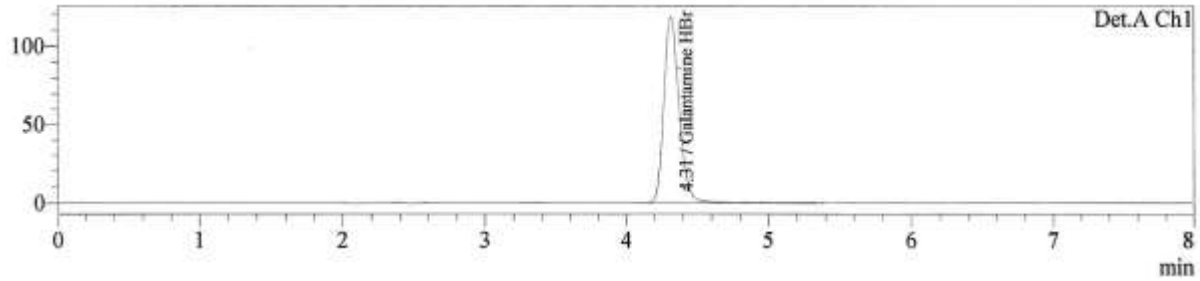


Fig .No.19: Chromatogram showing accuracy-120% injection

Table.No-4-Accuracy of galantamine

Concentration (%)	Added concentration (mg/mL)	Area	Recovered concentration (mg/mL)	Recovered (%)	Average	SD	%RSD
25	0.0067	211771	0.0068	101.5	100.5	0.870	0.9
	0.0068	212056	0.0068	100.0			
	0.0068	211057	0.0068	100.0			
50	0.0121	377700	0.0121	100.0	99.7	0.460	0.5
	0.0121	378056	0.0121	100.0			
	0.0121	376133	0.0120	99.2			
80	0.0190	588564	0.0188	98.9	98.8	0.320	0.3
	0.0191	589079	0.0189	99.0			
	0.0191	588238	0.0188	98.4			

100	0.0243	756311	0.0242	99.6	99.6	0.000	0.0
	0.0243	756468	0.0242	99.6			
	0.0243	757270	0.0243	99.6			
120	0.0283	881278	0.0282	99.6	99.4	0.400	0.4
	0.0284	882513	0.0283	99.6			
	0.0285	881570	0.0282	98.9			
AVG					99.6		
SD					0.61		
%RSD					0.6		

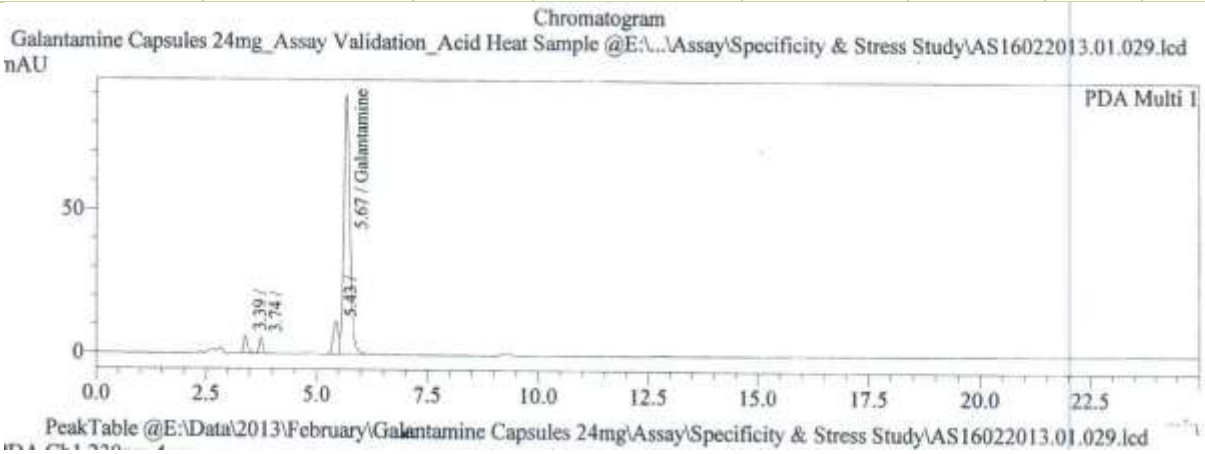


Fig .No.20: Sample treated with 5N HCl

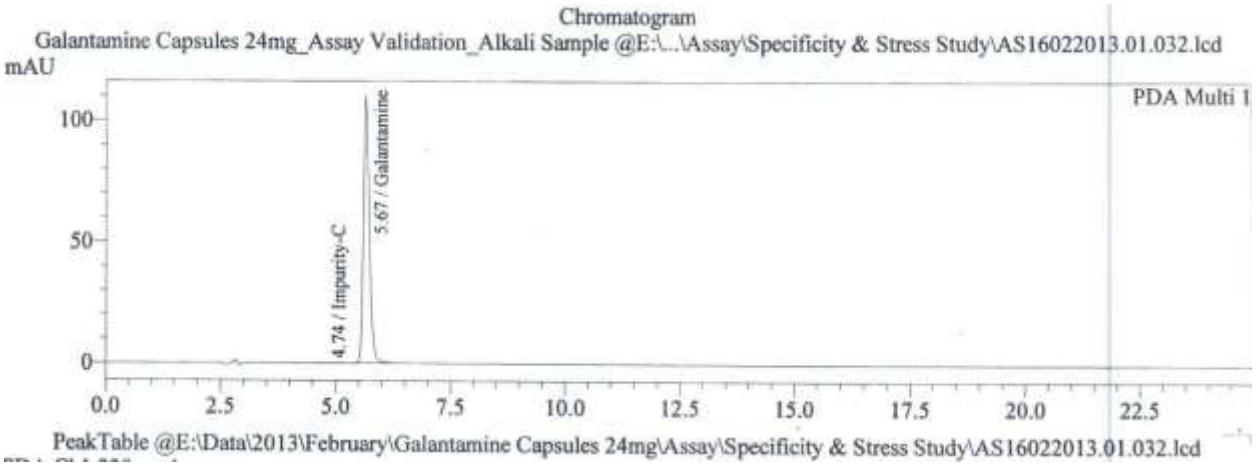


Fig: No-21.Sample treated with 5N NaOH

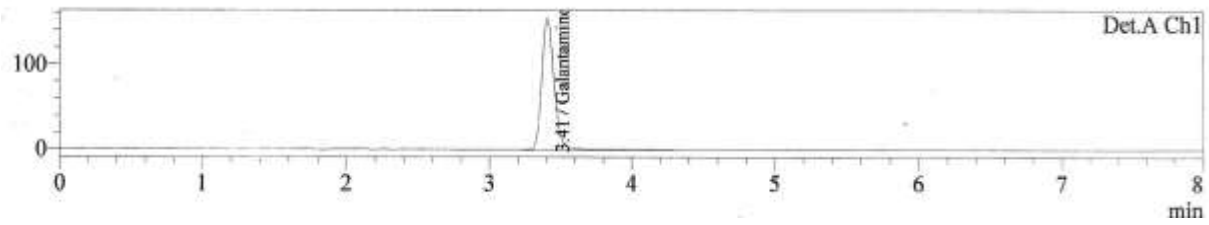


Fig .No.22: Robustness: High Flow

Table.No.5.System Suitability and System Performance Results for Robustness

Robustness High Flow (1.1 ml/min)							
Sample	Area	Theoritical Plates	Tailing Factor	%RSD	System Performance (%)	% Assay	Pass/Fail
1	912074	6647	1.2	0.1	0.4	100.7	Pass

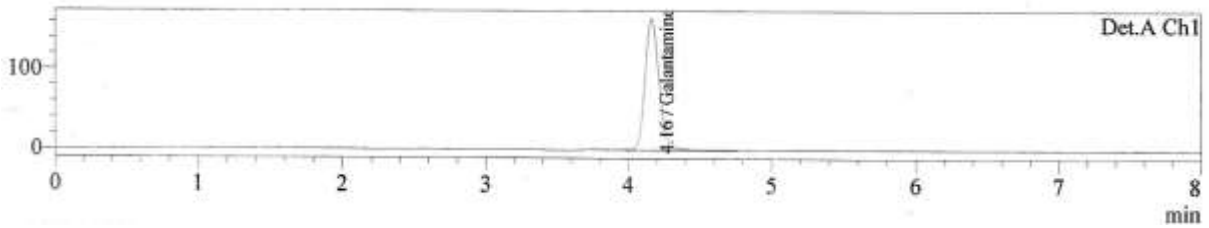


Fig .No.23: Robustness: Low Flow

Table.6.System Suitability and System Performance Results for Robustness

Robustness Low Flow (0.9 ml/min)							
Sample	Area	Theoritical Plates	Tailing Factor	%RSD	SystemPerformance (%)	% Assay	Pass/Fail
1	1114215	7863	1.2	0.2	0.1	100.5	Pass

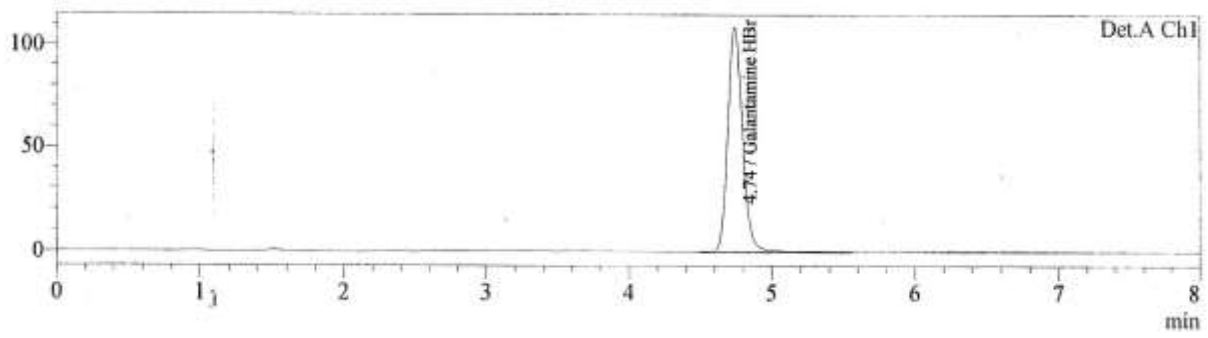


Fig .No.24: High buffer and low solvent

Table.No.7 System Suitability and System Performance Results for Robustness

Robustness High Buffer and Low solvent							
Sample	Area	Theoritical Plates	Tailing Factor	%RSD	SystemPerformance (%)	% Assay	Pass/Fail
1	953399	9388	1.2	0.0	0.0	99.5	Pass

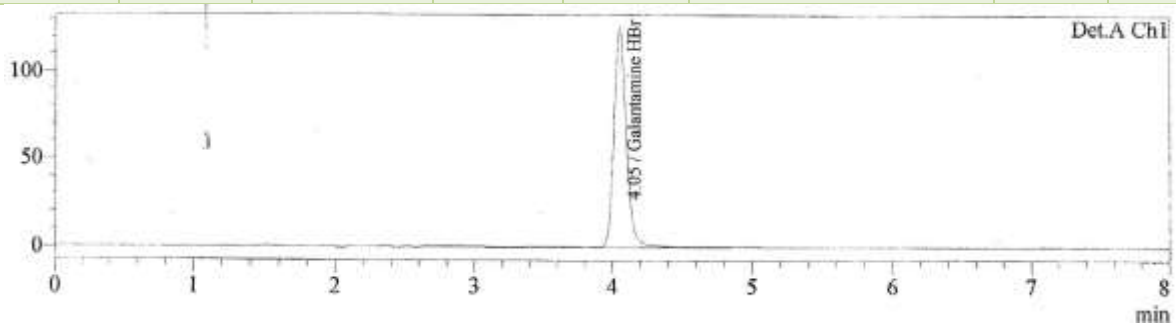


Fig .No.25: Low buffer and high solvent

Table.No.8: System Suitability and System Performance Results for Robustness

Robustness Low Buffer and High solvent							
Sample	Area	Theoritical Plates	Tailing Factor	%RSD	System Performance (%)	% Assay	Pass/Fail
1	962536	8830	1.2	0.1	0.0	99.6	Pass

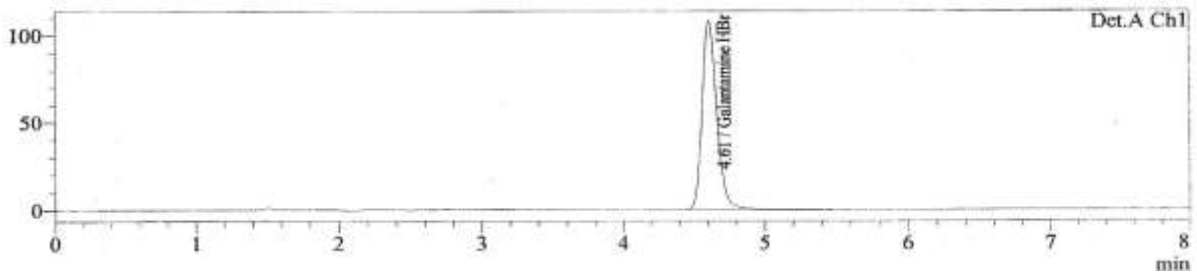


Fig .No.26: High ph.

Table.No.9: System Suitability and System Performance Results

Robustness High pH (6.70)							
Sample	Area	Theoritical Plates	Tailing Factor	%RSD	System Performance (%)	% Assay	Pass/Fail
1	1020767	8830	1.2	0.1	0.1	101.7	Pass

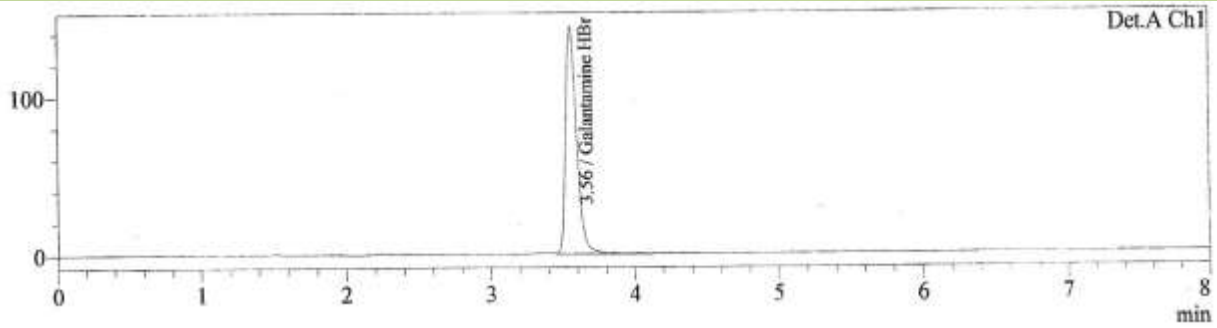


Fig .No.27: LOW pH:

Table.No.10: System Suitability and System Performance Results for Robustness

Robustness Low pH (6.30)							
Sample	Area	Theoritical Plates	TailingFactor	%RSD	System Performance(%)	%Assay	Pass/Fail
1	992735	9096	1.2	0.1	0.0	101.2	Pass

RESULTS AND DISCUSSION

Galantamine is an anti-alzeimers drug and is analysed by RP-HPLC method. From the results shown in table( ) the taken assay value of taken sample of galantamine HBr were found to be 99.69% .

From the linearity Table it was found that the drug obeys linearity within the concentration range of Galantamine HBr.

From the results shown in specificity it was observed that sample showed much degradation with addition of 0.1N HCL and least degraded with 0.1N NaOH and heat treatment.

From the results shown in accuracy it was found that the mean percentage recovery values of pure drug were in between 99.43% to 99.2% for Artemether and Lumefantrine respectively, and as these results are within the range of limits which indicates that the method was accurate.

From the results shown in precision it was found that % RSD is not more than 2%; as these results are within the range of limits which indicates that the proposed method has good reproducibility. The results are good for both method precession and system precession.

From the results shown in ruggedness, as it was performed by the two people the %RSD was found within the limits i.e.,should not more than 2.So the method obeys ruggedness.

From the results shown in robustness, the system suitability parameters were within the limit at all variable conditions. So the method was robust.

SUMMARY AND CONCLUSION

Development of new analytical methods for the determination of drugs in pharmaceutical dosage forms is more important in pharmacokinetic, toxicological and biological studies. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. The pharmaceutical industry is under increased scrutiny from the government and the public interested groups to contain costs and at consistently deliver to market safe, efficacious product that fulfill unmet medical needs. The pharmaceutical analyst plays a major rule in assuring identity, safety, efficacy, purity, and quality of a drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. The commonly used tests of pharmaceutical analysis generally entail compendia testing method development, setting specifications, and method validation. Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products. New methods are now being developed

with a great deal of consideration to worldwide harmonization. As a result, new products can be assured to have comparable quality and can be brought to international markets faster.

Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs.

Among the several techniques [HPLC, GC, NMR, MS, spectrophotometry IR] available for the assay of drugs, the author had chosen spectrophotometry, HPLC and fluorescence spectrometry. The visible spectrophotometric technique is simple and less expensive. The selectivity and sensitivity of the visible spectrophotometric method depends only on the nature of the chemical reactions involved in color development.

HPLC is a major tool for the qualitative and quantitative analysis of drugs and pharmaceuticals, chemical and biological materials and also plays very important role in pharmacokinetics studies. HPLC technique has been regarded as the best among various instrumental ones in spite of its heavy cost and maintenance problems.

In the present investigation the author had selected three Galantamine HBr . For this very few analytical methods were reported and hence there is wide scope for the development of new analytical methods for their quantitative analysis.

Impurity profiling is very important during the synthesis of drug substances and manufacture of dosage forms, as it can provide crucial data regarding the toxicity, safety, various limits of detection, and limits of quantitation, of several organic and inorganic impurities, usually accompany with bulk drugs and finished products. So, for GAL the author had developed HPLC method for determination of related impurities present in the GAL capsules. The three impurities reported IMP-A, IMP-C IMP-D, are well separated. Other impurities are not necessary to identify and quantify because the levels are within the limits according to the regulatory guidelines.

The thesis describes that the proposed methods can be used as alternative methods to reported ones and provides a wide choice for routine determination of the above mentioned drugs. ***Thus the purpose of the present investigation was successfully achieved.***

BIBLIOGRAPHY

1. These sources were u Douglas A. Skoog, F.James Holler Principles of Instrumental analysis 5yh edition.

2. Goodman, L.S and Gilman, A.G., The Pharmacological Basis of Therapeutics, 9th Edn. By Hardman, J.G., Limbard, L.E., Editors in chief, McGraw – Hill, 1996.

3. William O. Foye, Edt., Principles of Medicinal Chemistry, 3rd Edn., Varghese, Bombay, 1989

4. Lachman, L., Lieberman, H.A., and Kaning, J.L., The Theory and Practice of Industrial Pharmacy, 2nd Edition, London.

5. Bauman, R.P., Absorption spectroscopy, John Wiley and Sons, New York, 1962, Chaprer 9.

6. David G. Watson, Pharmaceutical Analysis, 1st edition, Churchill Livingstone,

Harcourt Publishers limited, 1999.

7. Roger E. Schirmer Modern methods of Pharmaceutical Analysis 2nd edition Volume 1.

8. Beckett, A.H., Stenlake, J.B., Practical Pharmaceutical Chemistry, Vol. I & II, CBS Publishers and Distributors, New Delhi, 1986.

9. Kirkbright, G.P., Talanta, 1996,13, 1.

10. Denny R.C., "A Dictionery of Spectroscopy" 1973, Mac Millon, London.

11. Sandell, E.B "Colorimetric determination of traces of metals", 1950, Interscience, New York.

12. Ringbom, A., Z. Anal.Chem, 1938, 115, 332.

13. Baur E.L "A Statistical Manual for Chemists", 1960, Academic Press, London.", 1960, Academic Press, London.

14. M .Narayana Reddy.et.al., THE EASTREN PHARMACIST, March-2008 .

15. C.S.R Lakshmi, M.Narayana Reddy.et.al., THE EASTREN PHARMACIST, July-1999.

16. M .Narayana Reddy, G Devela Rao. et.al., THE EASTREN PHARMACIST, August-1993 .