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Analytical Development And Validation Of Stability-Indicating Method For Estimation Of Amantadine In Pharmaceutical Dosage Forms By Using RP–UPLC

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Abstract

A simple, Accurate, precise method was developed for the estimation of the Amantadine in bulk and pharmaceutical dosage form. Chromatogram was run through ACQUITY UPLC BEH C18 Column, 1.7 µm, 2.1 mm X 50 mm. Mobile phase containing 0.1% AmmoniumFormate: Methanol taken in the ratio 73.6 (%v/v) and 26.3 was pumped through column at a flow rate of 0.28 ml/min. Temperature was maintained at 29.21°C. Optimized wavelength selected was ACQUITY TUV ChA 219 nm. Retention time of Amantadine was found to be 1.814 min. %RSD of the Amantadine was found to be 0.4%. %Recovery was obtained as 99.94% for Amantadine. LOD, LOQ values obtained from regression equations of Amantadine were 0.05, 0.15. Regression equation of Amantadine is y = 52995x + 2524.1. with regression coefficient value is found to be 0.99. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Amantadine, Method development, RP-UPLC, System Suitability, Stability indicating

1. Introduction

Most medications in multicomponent dosage forms may be analysed by the UPLC system thanks to its many benefits, including speed, specificity, consistency, accuracy, precision, and simplicity of automation. The UPLC method saves repeating extraction and isolation procedures. There are several modes of differentiation in UPLC. These are Size Exclusion Chromatography, Chromatography of Reversed Phase Ion Phase, Chromatography of Affinity, Normal Phase Mode, and Inverted Phase Mode.

The effectiveness and safety of a medicine are significantly influenced by the quality of the drug. For customers to have access to safe and effective medicinal formulations, quality assurance and control of pharmaceutical and chemical formulations are crucial. Hence When determining whether a chemical is suitable for use in patients, analysis of both the pure drug material and its pharmaceutical dose forms is crucial. The calibre of

the processes used to generate the data are what determines the calibre of the analytical data. To ensure that pharmaceuticals and their formulations are legally certified by regulatory bodies, it is crucial to establish tough and reliable analytical procedures.[1].

UPLC:

UPLC is an emerging area of analytical separation science which retains the practicality and principles of UPLC while increasing the overall interlaced attributes of speed, sensitivity and resolution. Speed and peak capacity can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC by using fine particles. UPLC takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, sensitivity and superior resolution. In this article we explored the potential of UPLC to improve the analysis of the samples that are encountered during pharmaceutical development and manufacturing. Particular emphasis has been placed on determining whether UPLC can reduce analysis times without compromising the quantity and quality of the analytical data generated compared to UPLC.[2-5]

An antiviral that is used in the prophylactic or symptomatic treatment of influenza A. It is also used as an antiparkinsonian agent, to treat extrapyramidal reactions, and for postherpetic neuralgia. For the chemoprophylaxis, prophylaxis, and treatment of signs and symptoms of infection caused by various strains of influenza A virus. Also for the treatment of parkinsonism and drug-induced extrapyramidal reactions. It is chemically called as adamantan-1-amine [6].

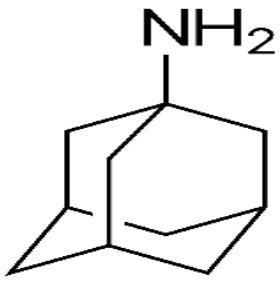

Figure1: Chemical structure of Amantadine

Experimental Work: [7-12]

2. Materials And Methods

Chemicals and reagents

Pure Amantadine was procured from Spectrum pharma lab (Hyderabad). Hydrochloric acid AR grade (HCL) and sodium hydroxide AR grade (NAOH) were obtained from rankem, India. Hydrogen Peroxide (H_2O_2) was purchased from Qauligens. Acetic acid AR grade was purchased from Fisher scientific, India and S.D. Fine chem Ltd. Respectively. Potassium dihydrogen orthophosphate and orthophosphoric acid were obtained from S.D. Fine chem Ltd and Merck India Pvt Ltd. Respectively. UPLC grade Acetonitrile (ACN) and methanol (MeOH) were purchased from Fischer scientific. UPLC grade water used throughout analysis was obtained from the Merck milli-Q water purification unit.

Apparatus and Equipment

- UPLC studies were carried out on WATERS UPLC 2965 SYSTEM with a Photo diode array detector (PDA) set at 220 nm for uv detection. columns, viz; Agilent C18 (150×4.6 mm, 5 µm), Discovery C18(150*4.6mm,5 µm), Zodiac (150*4.6mm,5 µm) ,BDS (150*4.6mm,5 µm) and Phenomenex (150*4.6mm,5 µm)column were utilized in the study. Design Expert® (11.0.0) modeling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for generation of contour plots and 3D space.
- pH meter (Eutech instruments pH tutor, pH meter, India) was used to check the pH of all solutions.
- Other equipment sonicator (ePEI ultrasonic generator), Analytical balance (Mettler Toledo), vortex meter (IKA Vortex), Hot air oven (Yorco scientific).

Preparation of buffer

0.01N Potassium dihyrogen ortho phosphate

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution

0.1% Ortho phosphoric acid buffer

1ML of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

Preparation of drug solution

Preparation of Standard stock solutions: Accurately weighed 10mg of Amantadine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Amantadine)

Preparation of Standard working solution (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Amantadine).

Preparation of Sample stock solutions: 10 mg of tablet were taken and calculate the average weight of each tablet and Weight equivalent to 1 tablet was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (1000µg/ml of Amantadine)

Preparation of Sample working solution: From the filtered solution 0.2 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent. (20µg/ml of Amantadine).

Methodology for Validation Parameters:[13-15]

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Amantadine (20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions: Accurately weighed 10mg of Amantadine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Amantadine)

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Preparation of Sample working solution: From the filtered solution 0.2 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent. (20µg/ml of Amantadine).

Linearity:

Preparation of Standard stock solutions: Accurately weighed 10mg of Amantadine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Amantadine)

25% Standard solution: 0.25ml from standard stock solution was pipette out and made up to 10ml. (5µg/ml of Amantadine)

50% Standard solution: 0.5ml from standard stock solution stock solutions was pipetted out and made up to 10ml. (10µg/ml of Amantadine)

75% Standard solution: 0.75ml from standard stock solution stock solutions was pipetted out and made up to 10ml. (15µg/ml of Amantadine,)

100% Standard solution: 1.0ml from standard stock solution stock solutions was pipetted out and made up to 10ml. (20µg/ml of Amantadine)

125% Standard solution: 1.25ml from standard stock solution stock solutions was pipetted out and made up to 10ml. (25µg/ml of Amantadine)

150% Standard solution: 1.5ml from standard stock solution stock solutions was pipettede out and made up to 10ml. (30µg/ml of Amantadine)

Accuracy:
Preparation of Standard stock solutions: Accurately weighed 10mg of Amantadine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Amantadine)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:
The % Recovery for each level should be between 98.0 to 102
Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.
Robustness conditions like Flow minus (0.1ml/min), Flow plus (0.3ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml Standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.25ml Amantadine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.9ml Amantadine of, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies: [16-19]
Oxidation:
To 1 ml of stock solution of Amantadine, 1 ml of 20% hydrogen peroxide (H2O2)was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study,the resultant solution was diluted to obtain 20µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:
To 1 ml of stocks solution Amantadine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.The resultant solution was diluted to obtain 2 0 µg/ml solution and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:
To 1 ml of stock solution Amantadine, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:
The standard drug solution was placed in oven at105°C for 1h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 20µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the 200µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1hrs or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxingthedruginwaterfor1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Initial UPLC runs of Amantadine
Initial UPLC runs of Amantadine of 20 µg/mL concentration were performed using

- Different buffer viz, Potassium dihyrogen ortho phosphate and Ortho phosphoric acid.
- Different organic modifier viz, acetonitrile and methanol
- Different columns such as Symmetry C18 (150×4.6 mm, 5 µm), Agilent C18 (150×4.6 mm, 5 µm), Discovery C18(150 ×4.6mm,5 µm), Zodiac (150×4.6mm,5 µm), BDS (150×4.6mm,5 µm) and Phenomenex (150 ×4.6mm,5 µm) column.

3. Result and Discussion

Authentication by Amantadine API UV-VIS spectra
After scanning from 400 to 200nm in UV-VIS spectrophotometer, Amantadine was showed absorption maxima at 219.0 nm in Methanol.

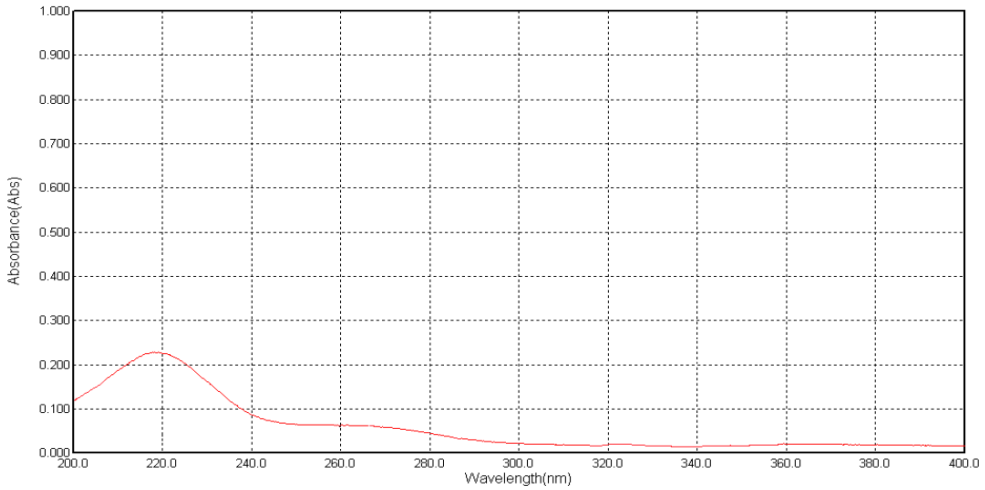

Figure 2: UV Spectrum for the Amantadine

Parameter selection

Various preliminary UPLC trials were carried out for selection of Column and organic modifier. The choice of C18 column based on the preliminary investigation was done using Symmetry C18 (150×4.6 mm, 5µm), Azilent C18 (150×4.6 mm, 5µm), Discovery (150×4.6 mm, 5µm) and BDS (150×4.6 mm, 5 µm) columns. Symmetry C18 column having less tailing, higher theoretical plate and good shape of drug peak as compare to the Azilent, BDS and Discovery column. Selection of a suitable organic modifier is also important to get better selectivity with adequate separation of all analytes. Commonly used organic solvents for the reversed phase UPLC include Acetonitrile and Methanol from that trials Acetonitrile showed to be an ideal and suitable organic modifier compared to acetonitrile, because Amantadine was solubilized in acetonitrile compare to methanol. Therefore, acetonitrile was selected and finalized as the organic modifier for further optimization study.

Optimized UPLC method for Amantadine:
Chromatographic conditions:

Columm dimensions: ACQUITY UPLC BEH C18 Column, 1.7 µm, 2.1 mm X 50 mm

Mobile Phase: 0.1% Ammonium Formate: Methanol (73.6 V/V: 26.3 V/V)

Flow rate: 0.28 ml/min

Temperature: 29.12ºC

UV Detector with Wave Length: ACQUITY TUV ChA 219 nm

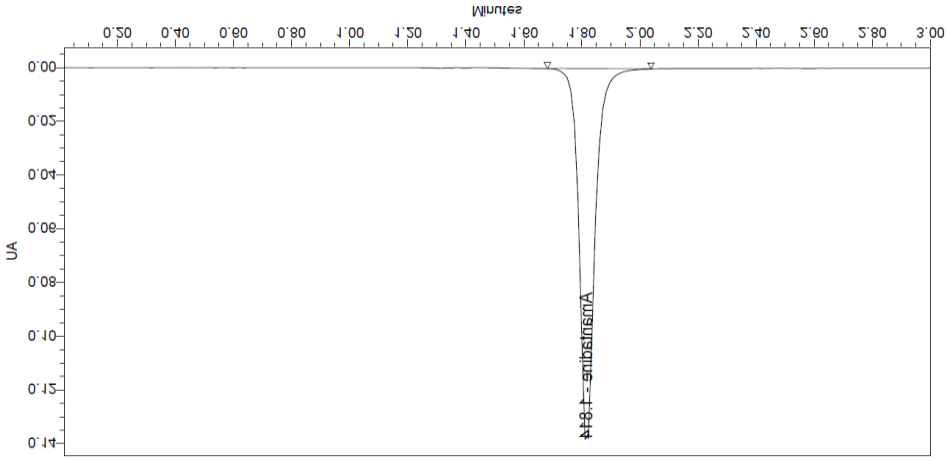

Figure 3: optimized chromatogram for Amantadine

Table 1: System suitability for Amantadine

S.No	Name of the Drug	Retention tine	Area	USP Plate Count	USP Tailing
1	Amantadine	1.184	1045038	8017	1.05

Here system suitability values obtained for the for Camylofin and Mefenamic Acid found to be with in the limitations therefore the following Parameters were used for the method validation as per the USP guidelines,

Method validation
Validation:

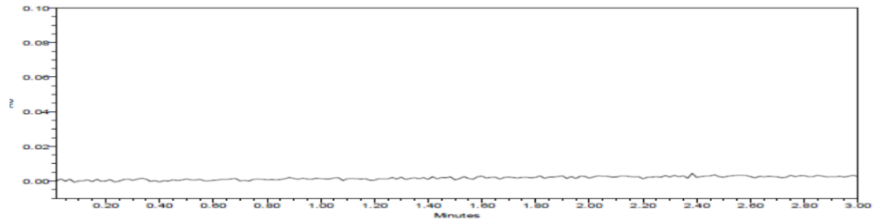

Figure4 blank Chromatogram

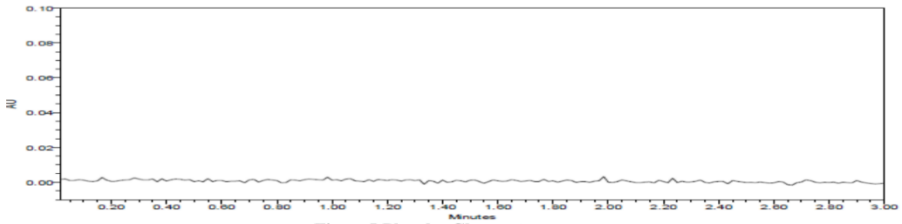

Figure5 Placebo Chromatogram

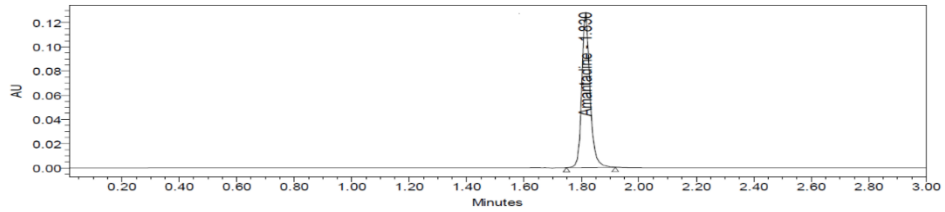

Figure5: Standard Chromatogram of Amantidine

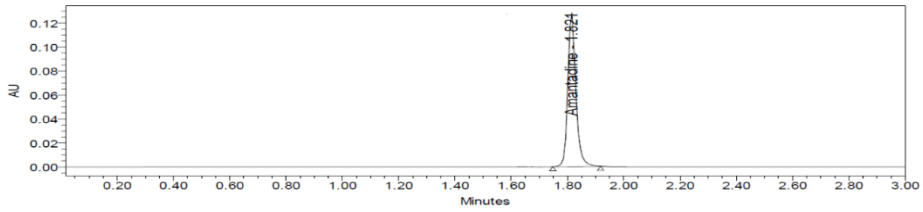

Figure6 Sample Chromatogram of Amantidine

Retention time of Amantadine was 1.821 min. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Precision:

System Precision: Six working sample solutions of 20ppm are injected and the % Amount found was calculated and %RSD was found to be 0.4 and chromatogram was shown in fig

Table 2 System Precision data

S.No	Peak Area
1	1071324
2	1066647
3	1069843
4	1075862
5	1068874
6	1062264
AVG	1069136
STDEV	4561.0
%RSD	0.4

Method precision: Six working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 1.0 and chromatogram was shown in fig 6.3.

Table 3 Method precision data

S.No	Peak Area
1	1074976
2	1059172
3	1065933
4	1058407
5	1068799
6	1044806
AVG	1062016
STDEV	10455.7
%RSD	1.0

Intermediate precision: Six working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 1.2 and chromatogram was shown in fig 6.3.

Table 4 Intermediate precision data

S.No	Peak Area
1	1014552
2	1021286
3	1016125
4	1032367
5	1005632
6	1038785
AVG	1021458
STDEV	12213.6
%RSD	1.2

LINEARITY:

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 5ppm to 30ppm of Amantadine. Plot a graph to concentration versus peak area. Slope obtained was y = 52995x + 2524.1 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Fig 6.15.

Table 5 Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	5	266684
50	10	532281
75	15	805170
100	20	1057956
125	25	1333080
150	30	1586923

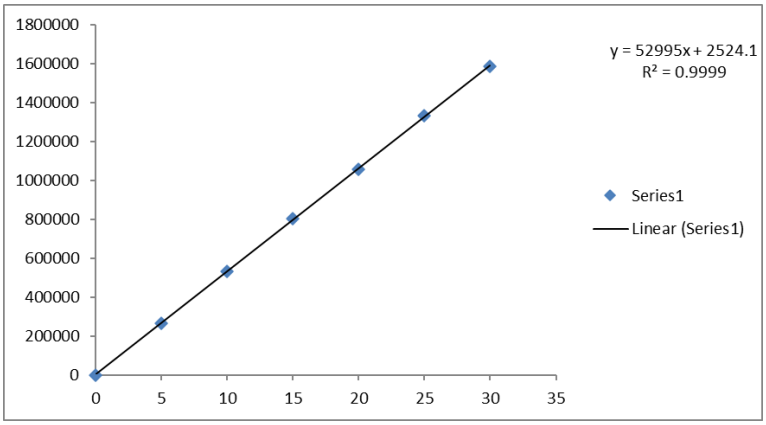

Figure 7 Linearity Graph for Amantidine

Accuracy: Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 99.94%. And chromatograms were shown in fig 6.11-6.13.

Table 6. Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	**10**	**9.95**	**99.47**	**100.02%**
	10	**9.95**	**99.45**	
	10	**9.98**	**99.76**	
100%	**20**	**20.23**	**101.13**	
	20	**19.87**	**99.33**	
	20	**19.97**	**99.83**	
150%	**30**	**30.04**	**100.14**	
	30	**29.99**	**99.96**	
	30	**30.11**	**100.38**	

LOD: Detection limit of the Amantadine in this method was found to be 0.05µg/ml.
LOQ: Quantification limit of the Amantadine in this method was found to be 0.15µg/ml.
Robustness: Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated

Table 7 Robustness Data

Parameter	%RSD
Flow Rate Minus	0.5
Flow rate Plus	0.7
Mobile Phase Composition /Concentration Minus	0.2
Mobile Phase Composition /Concentration Plus	0.3
Temperature minus	0.6
Temperature plus	0.6

Assay Of Marketed Formulation
The Marketed formulation of brand @ Gocovri was taken for analysis with composition of the 100 mg and the Standard solution and sample solution of the Concentration 200µg/ml were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.
Calculation Formula: Assay (%w/w)

$$\frac{\textbf{Sample Area} \times \textbf{test dilition} \times \textbf{Sample Purity} \times 1}{\textbf{Standard Area} \times \textbf{Standardd Dilution} \times \textbf{Standard Purity} \times \textbf{Label Claim}} \times 100$$

Dilution factor for Amantadine: 100
Label claim:100mg

Table 8 Assay of Formulation of Amantadine

S. No	Area of Standard	Area of Sample	%Assay
1	1071324	1074976	100.35
2	1066647	1059172	98.87
3.	1069843	1065933	99.50
4.	1075862	1058407	98.80
5.	1068874	1068799	99.77
6.	1062264	1044806	97.53
AVG	1069136	1074976	99.14
STDEV	4561.0	1059172	0.98
%RSD	0.4	1065933	0.98

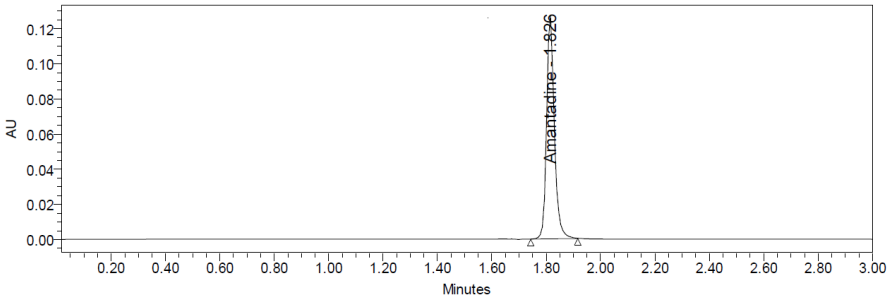

Figure 8 Assay chromatigram foir standard drug Amantidine

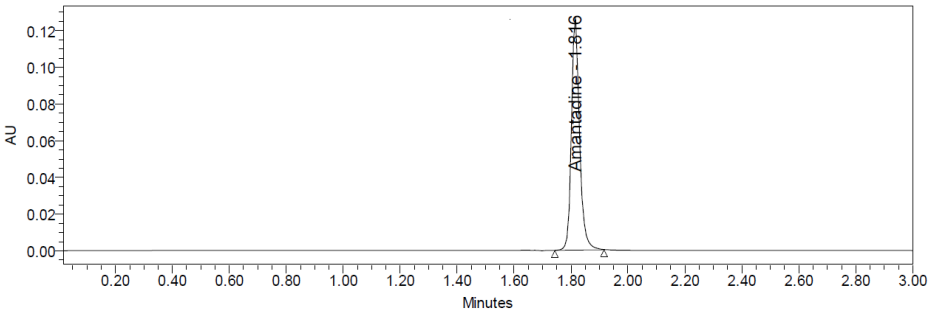

Figure 9 Assay chromatogram of Marketed Sample Amantadine

Table 9: Summary of the Results for Amantadine

Parameters	Amantadine	LIMIT
Linearity Concentration of Amantadine Range (µg/ml)	5-30µg/ml	
Regression coefficient	0.999	
Slope(m)	52995	
Intercept(c)	2524.1	
Regression equation (Y=mx+c)	y = 52995x + 2524.1	
Assay (% mean assay)	99.94%	90-110%
Specificity	Specific	No interference of any peak
System precision %RSD	0.4	NMT 2.0%
Method precision %RSD	1.0	NMT 2.0%

Accuracy % recovery		99.94%	98-102%
LOD		0.05/ml	NMT 3 µg/ml
LOQ		0.15µg/ml	NMT 10µg/ml
Robustness	Flow Rate Minus	0.5	%RSD NMT 2.0
	Flow Rate Plus	0.7	
	Mobile Phase Concentration Minus	0.2	
	Mobile Phase Concentration Plus	0.3	
	Temperature Minus	0.6	
	Temperature Plus	0.6	

The present study was aimed at developing a sensitive, precise, and accurate stability indicating UPLC method for the analysis of Amantadine in bulk drug and pharmaceutical dosage forms by using QbD approach using Design Expert® software. The central composite design experimental design describes the interrelationships of mobile phase and pH at three different level and responses to be observed were retention time, theoretical plates, and peak asymmetry with the help of the Design Expert 11.0 version. Here, a better understanding of the factors that influence chromatographic separation with greater confidence in the ability of the developed UPLC method to meet their intended purposes is done. The QbD approach to analytical method development was used for better understanding of method variables with different levels. it is a faster way of developing method, which helps choosing much better method condition during the development process through design space. The validation results confirmed the usefulness of the method. The method was found to be precise, accurate and linear, whereas the stress degradation studies identified the potential degradation products which can form during the shelf life of the product. In order to affect proper elution of the component peaks, mixtures of Acetonitrile with Phosphate Buffer in different combinations were tested as mobile phase on a non-polar ACQUITY UPLC BEH C18 Column, 1.7 µm, 2.1 mm X 50 mm. A mixture of Ammonium Formate(73.6) (pH 3.6) and Methanol (26.3 v/v) was proved to be the most suitable of the combination since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time for Amantadine was at 0. ± 10% min respectively injected at a flow rate of 0.28mL/min. The detection wave length was fixed at 219.0 nm as the peak areas were consistent and reproducible over a run time of 3.00 minutes. The injection volume was optimized at 0.2 µL. The method obeyed linearity in the range of 5-30 µg/mL for Amantadine respectively as observed from the linearity curves. The regression equations for Amantadine were found to be y = 52995x + 2524.1 (R2 = 0.999) respectively. The specificity of the method was established by studying the Amantadine peaks in the presence of excipients. The commonly present excipients did not pose any interference at the retention time of the drug as they were not identified. The repeatability and intermediate precision were studied by analyzing the sample solutions of Amantadine. The low coefficients of variation obtained in the intraday (1.0 %) and inter day precision (1.2 %) study are indicative of the precision of the method. High recovery values for Amantadine (99.94%) obtained from the analysis of dosage forms by the proposed method indicates the accuracy of the method. The deliberate changes in the method (flow rate and temperature) have not much affected the peak tailing, theoretical plates, resolution, and percent assay. This indicates that the present method is robust (% RSD < 2). The limit of detection and limit of quantitation for Amantadine were found to be 0.05 µg/mL and 0.15 µg/mL respectively. The low values of LOD and LOQ as obtained by the proposed method indicate the sensitivity of the method. The developed UPLC method was applied for the analysis of Amantadine in marketed formulations. The marketed dosage form was found to contain an average of 99.94 ± 10 % w/v of Amantadine as stated on the label claim. The absence of additional peaks indicates non-interference of common excipients used in the Sample preparations. The proposed UPLC

method was also applied for the forced degradation studies on Amantadine under a variety of conditions like acid and base hydrolysis, oxidation, and heat and photo stability. The drugs were found to be stable except in acidic, Basic, Peroxide stress conditions. The drug peaks in these degradations were found to be homogenous and no other peaks merged. No major degradants were found in Neutral stress, photo stability and Thermal degradation studies. As the developed method could effectively separate Amantadine from the degradants, it can be employed as a stability indicating assay. System suitability testing was performed prior to the study of each validation parameter and the verified parameters like tailing factor (< 2.0), resolution (> 8.2), column efficiency (> 2000) and repeatability (% RSD < 2) ensured that the equipment, electronics, and analytical operations for the samples analysed could be constituted as an integral system that can be evaluated as a whole.

4. Conclusion:

The developed UPLC method is specific, sensitive, precise, accurate and stability indicating. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Amantadine in bulk drugs and pharmaceutical dosage forms without any interference from degradants or excipients. This method can also be used for the regular quality control analysis of Amantadine, Results which were obtained from the validation of developed analytical method were within limit as per ICH guidelines.

5. References:

1. A Handbook of modern pharmaceutical analysis, Separation Science and Technology. In Ahuja, S.; Scypinski, S., Eds. Academic Press, USA: 2001; Vol.3,p 2.
2. Handbook of modern pharmaceutical analysis, Separation Science and Technology. In Ahuja, S.; Scypinski, S., Eds. Academic Press, USA: 2001; Vol.3, p 383.
3. Handbook of modern pharmaceutical analysis, Separation Science and Technology. In Ahuja, S.; Scypinski, S., Eds. Academic Press, USA: 2001; Vol.3, p 343.
4. Beckett, A. H.; Stenlake, J. B. Practical pharmaceutical chemistry. In CBS publishers and distributors, New Delhi: 1997; Vol.2, p 163.
5. http://bheem.hubpages.com/hub/UPLC-detector-types
6. https://pubchem.ncbi.nlm.nih.gov/compound/Amantadine
7. ICH Q2A and 2B , Validation of Analytical Procedures: Definitions and Terminology, Geneva, 1995, in 2005 incorporated in Q2(R1)
8. Guideline on general principal of validation. U.S. Department of Health and Human Services, Food and Drug Administration, 1987.
9. USP 32 – NF 27, General Chapter 1226, Verification of Compendial Methods,2009
10. ICH Harmonised Tripartite Guideline Validation of Analytical Procedures:Text and Methodology Q2(R1)
11. USP 32 – NF 27, General Chapter 621, Chromatography, 2009
12. El-Shorbagy HI, Mohamed MA, El-Gindy A, Hadad GM, Belal F. Development of UPLC method for simultaneous assay of some COVID-19 drugs utilizing novel instrumental standard addition and factorial design. Scientific Reports. 2023 Apr 4;13(1):5466.
13. Kannaiah KP, Sugumaran A, Chanduluru HK. Simultaneous estimation of crotamiton and hydrocortisone by RP-UPLC using green analytical principles embedded analytical quality by design (AQbD) method. Microchemical Journal. 2023 Jan 1;184:108166.
14. Narla Mahendra Kumar*1 , Chennu MM Prasada Rao2 "QBD Based Analytical Development And Validation Of Stability-Indicating Method For Estimation Of Molnupiravir In Pharmaceutical Doses From RP–UPLC" Eur. Chem. Bull. 2023, 12 (S3) 3105– 3118 doi: 10.31838/ecb/2023.12.s3.3782023.22/05/2023
15. Akter MR, Hossain MS, Alam KK, Rafiquzzaman M. Development and Validation of RP-HPLC Method for the Determination of Anticancer Drug Brigatinib. GSC Biological and Pharmaceutical Sciences. 2023;23(3):030-41.
16. Bisen AC, Sanap SN, Biswas A, Agrawal S, Mishra A, Kumar M, Choudhury AD, Bhatta RS. A QbD-led simple and sensitive RP-UHPLC method for simultaneous determination of moxifloxacin, voriconazole, and pirfenidone: an application to pharmaceutical analysis. Biomedical Chromatography. 2023 Sep;37(9):e5681.