Stability Indicating Analytical Method Development And Validation Of Safinamide In Bulk And Its Marketed Pharmaceutical Dosage Form By Uplc

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ABSTRACT

A new analytical simple, accurate, precise, sensitive, rapid Ultra Performance Liquid chromatography (UPLC) method has been developed and validated for determination of Safinamide in bulk and its marketed pharmaceutical dosage form. Chromatographic separation was achieved on a Endeversil C_{18} ODS (2.1mm x 50mm, 3µm) column, by a mobile phase which consisted of 70% buffer (0.1% Octa sulphonic acid) and 30% Methanol taken in the ratio of 70:30% v/v, maintained pH to 3.0 with ortho phosphoric acid solution along with a flow rate of 0.2 ml/min. The detection wavelength was set at 254 nm. Safinamide was subjected to different stress conditions like acid, alkali, and peroxide, thermal and checked for its specificity, degradation & stability. The method was linear (r = 0.999) at a concentration range of 6-14 µg/ml. The intra and inter day precisions were satisfactory; the relative standard deviations did not exceed 2%. The accuracy of the method was proved; the mean recovery of Safinamide was 98.0-102.0%. The limit of detection and limit of quantitation of Safinamide was found to be 0.6030 µg/ml and 1.8273µg/ml respectively. The method met the ICH/FDA regulatory requirements. The results demonstrated that the method can be applied successfully for routine use in quality control industry and laboratories.

Key Words: Safinamide, UPLC, Accuracy, Precision, Linearity, ICH Guidelines.

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INTRODUCTION

Safinamide is an inhibitor of monoamine oxidase used as adjunctive therapy in combination with levodopa and carbidopa in the management of Parkinson's disease. Safinamide[1] has been associated with a low rate of serum enzyme elevations during treatment, but has not been linked to instances of clinically apparent acute liver injury. The mechanism of action of Safinamide illustrate that it is a Monoamine Oxidase-B Inhibitor, and Breast Cancer Resistance Protein Inhibitor. Safinamide is a unique molecule with multiple mechanisms of action and a very high therapeutic index. It combines potent, selective, and reversible inhibition of MAO-B with blockade of voltage-dependent Na+ and Ca2+ channels and inhibition of glutamate release. Safinamide[2] has neuroprotective and neurorescuing effects in MPTP-treated mice, in the rat kainic acid, and in the gerbil ischemia model. Safinamide is absorbed quickly and nearly completely from the gut and reaches highest blood plasma concentrations after 1.8 to 2.8 hours. There is no relevant first-pass metabolism; total bioavailability is 95%. The substance is bound to plasma proteins to 88–90%. The metabolism is not well understood. The principal step is mediated by amidases which have not been identified, and produces Safinamide acid (NW-1153). Other relevant metabolites are O-debenzylated Safinamide (NW-1199), the N-dealkylated amine which is then oxidized to a carboxylic acid (NW-1689), and the glucuronide of the latter. In tests with liver microsomes, dealkylation seemed to be mediated by CYP3A4, but other CYP enzymes appear to be involved as well. Safinamide acid binds to the organic anion transporter 3 (OAT3), but this has probably no clinical relevance. Safinamide[3] itself transiently binds to ABCG2. No other transporter affinities have been found in preliminary studies. Safinamide is eliminated, mainly (>90%) in form of its metabolites, via the kidney, with an elimination half-life of 20 to 30 hours. Only 1.5% is found in the stool. The IUPAC Name of Safinamide (2S)-2-[[4-[(3-fluoro phenyl) methoxy] phenyl] methyl amino] propanamide. The Chemical Structure of Safinamide is as follows

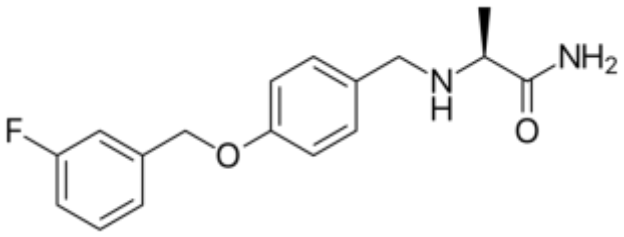

Fig-1: Chemical Structure of Safinamide

A few methods are reported[32-36] for the determination of Safinamide in bulk and marketed pharmaceutical formulations by HPLC, HPTLC, UV spectrophotometric, and colorimetric methods; however very few UPLC methods have been reported for its estimation till date. Hence an attempt has been made to develop and validate a rapid and sensitive UPLC method along with forced degradation studies for the estimation of Safinamide as recommended by the International Conference on Harmonization (ICH) guidelines[30-31]. The method was validated by parameters such as linearity, accuracy, precision, LOD, LOQ, robustness.

MATERIALS AND METHODS

Table-1: List of Instruments

SL. No	Instrument	Model
1	UPLC	Agilent 1290 Infinity II LC System
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table-2: Chemicals Used

SL. No	Chemical	Company Name
1	Safinamide	Janssen Pharmaceutical
2	KH_2PO_4	FINER chemical LTD
3	UPLC grade water	LICHROSOLV (MERCK)
4	UPLC grade Methanol	LICHROSOLV (MERCK)
5	UPLC grade Acetonitrile	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

Preparation of Buffer and Mobile Phase:

Preparation of 0.1% Octa sulphonic acid:

Accurately weighed 1 grams of Octa sulphonic acid was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with UPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of Mobile Phase:

Accurately measured 700 ml (70%) of above buffer and 300 ml of Acetonitrile UPLC (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration[4].

Diluent Preparation:

The Mobile phase[5] was used as the diluent.

Preparation of the Safinamide Standard & Sample Solution:

Standard Solution Preparation:

Accurately weighed and transfered 10mg of Safinamide working standard into a 10ml clean dry volumetric flask added about 7ml of diluent and sonicated to dissolve it completely, volume was made up to the mark with the same solvent. (Stock solution)

Further pipetted 1.0 ml of the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent. (100ppm of Safinamide)

Further pipetted 1.0 ml of the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent. (10ppm of Safinamide)

Sample Solution Preparation:

Accurately weighed 10 tablets crush in mortor and pestle and transfer equivalent to 10mg Safinamide (marketed formulation = 100mg of tablet Powder) sample into a 10ml clean dry

volumetric flask add about 7ml of diluent and sonicated it up to 15 mins to dissolve it completely, volume was made up to the mark with the same solvent. Then it was filtered through 0.45 micron Injection filter. (Stock solution)

Further pipetted 1.0ml of Safinamide from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (100ppm of Safinamide)

Further pipetted 1.0ml of Safinamide from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (10ppm of Safinamide)

Method Validation

The method[7-12] was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

System Suitability Parameters:

The chromato-graphic systems used for analysis must pass system suitability before going to start the experiment. At first UPLC system[13] is stabilized for 40 minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters[14] such as tailing factor (NMT 2.0), theoretical plate count (NLT 2000). The % RSD for the peak area of six replicate injections of Safinamide standard NMT 2.0. The parameters, such as tailing factor, % RSD, and theoretical plates, were studied.

Linearity

The linearity[15] of the method was established from the standard calibration curve graph[16] constructed at several concentration levels of 6.0-14.0 µg/ml for Safinamide. Each level was injected into the chromatographic system and measured the peak area. A graph was plotted of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculated the correlation coefficient.

Selectivity/Specificity

The Selectivity/specificity was evaluated by extracting different blank samples. The absence of interfering peaks at the same retention time of analytes was considered as evidence for selectivity/specificity[17].

Precision: The precision[18] of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was determined by performing six repeated analysis of the same working solution of Safinamide on the same day, under the same experimental conditions. The intermediate precision[19] of the method was assessed by carrying out the analysis on different days and also by another analyst performing the analysis in the same laboratory (between-analysts).

Accuracy: The accuracy[20] of a method is defined as the closeness of a measured value to the true value. The recovery studies[21] were carried out at 80%, 100% and 120% of the target level in the tablet in triplicate each in the presence of placebo.

Robustness: The robustness[22] was determined by analyzing the same sample under various conditions. The factors considered to be: variations in the flow rate, the organic ratio of the mobile phase, and pH. There were no significant changes in the chromatographic pattern[23] when the above modifications were made in the experimental conditions, showing that the method is robust. The % RSD of Safinamide should not be more than 2.0%.

LOD and LOQ: Limit of detection[24] is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation[25] is the lowest concentration of an analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated based on using following formulas, LOD = $3.3 \times \sigma/S$ and LOQ = $10 \times \sigma/S$, where σ is the deviation response, S is the slope of the calibration curve.

RESULTS AND DISCUSSION

Method Development

Wave length selection:

UV spectrum of 10µg/ml Safinamide in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength[26] selected as 254nm. At this wavelength both the drugs show good absorbance.

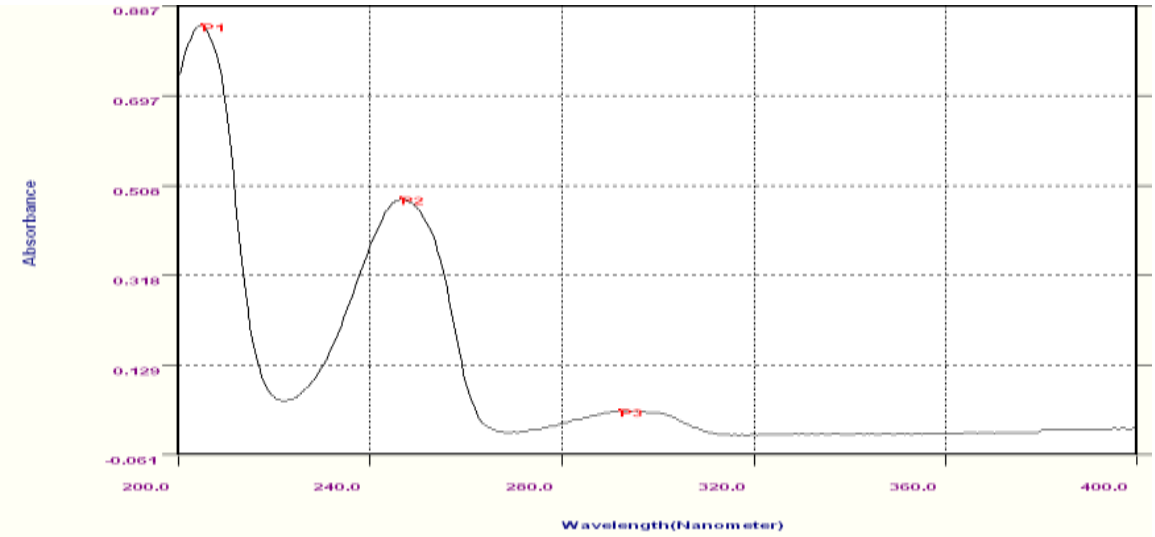

Fig-2: UV Spectrum of Safinamide at 254nm

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to 0.1% Octa sulphonic acid buffer (pH 3.0), Methanol in proportion 70: 30 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Hypersil column, lichrosorb, and Inertsil ODS column[27]. Endeversil ODS (2.1 x 50mm, 3⌈m) was found to be ideal as it gave good peak shape and resolution at 0.2 ml/min flow.

Optimized Chromatographic Conditions:

Instrument used	:	Agilent UPLC with auto sampler and PAD or detector.
Temperature	:	Ambient
Column	:	Endeversil C18 ODS (2.1 x 50mm, 3⌈m)
Buffer	:	0.1% Octa sulphonic acid
pH	:	3.0
Mobile phase	:	70% buffer 30% Methanol
Flow rate	:	0.2 ml per min
Wavelength	:	254 nm
Injection volume	:	2 ⌈l
Run time	:	10 min.

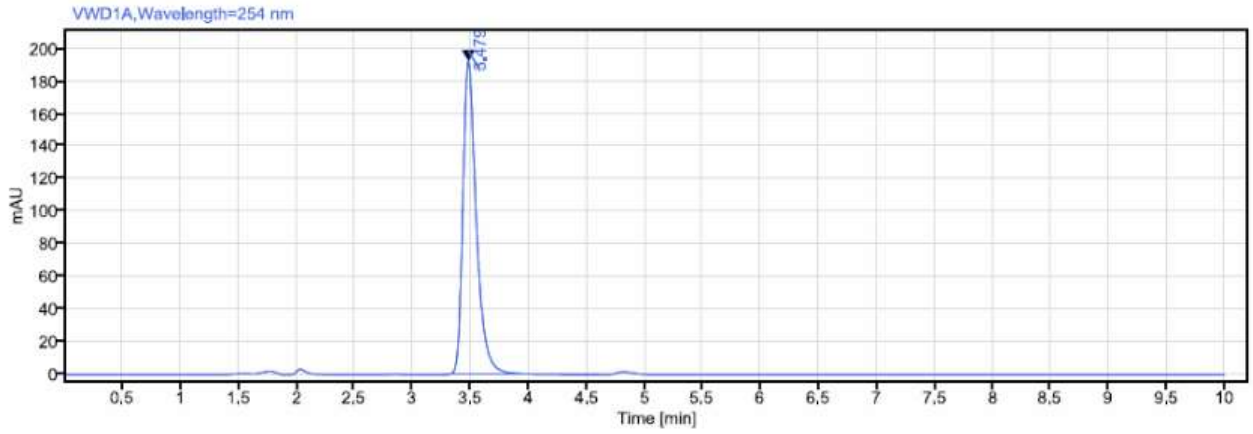

Fig-3: Optimized Chromatographic Condition

Method Validation

System Suitability:

Tailing factor for the peaks due to Safinamide in Standard solution should not be more than 2.0. Theoretical plates[28] for the Safinamide peaks in Standard solution should not be less than 2000.

Table-3: Results of System Suitability for Safinamide

Sl. No.	Peak Name	Retention Time (min)	Peak Area	Height (µV)	USP Plate Count	USP Tailing
1	Safinamide	3.493	616584	36528	5698.63	1.28
2	Safinamide	3.491	615989	36454	5674.25	1.29
3	Safinamide	3.491	616587	36251	5693.12	1.27
4	Safinamide	3.491	614543	36589	5624.89	1.28
5	Safinamide	3.489	615895	36785	5685.42	1.29
6	Safinamide	3.489	615985	36582	5698.37	1.27
Mean			615930.5			
Std. Dev.			747.11			
% RSD			0.12			

Precision:

Preparation of Stock Solution:

Accurately weighed and transfered 10mg of Safinamide working standard into a 10ml clean dry volumetric flask added about 7ml of diluent and sonicated to dissolve it completely, volume was made up to the mark with the same solvent. (Stock solution)

Further pipetted 1 ml of the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent. (100ppm of Safinamide)

Further pipette 1.0 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (10ppm of Safinamide)

Procedure:

The standard solution was injected for six times and measured the area for all six. Injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Method Precision:

Table-4: Results of Method Precision for Safinamide

Sl. No.	Peak name	Retention time(min)	Peak Area	Height (µV)	USP Plate Count	USP Tailing
1	Safinamide	3.481	615986	36524	5682.96	1.29
2	Safinamide	3.482	615387	35895	5687.47	1.28
3	Safinamide	3.482	616354	36487	5682.63	1.29
4	Safinamide	3.481	615842	36528	5689.42	1.30
5	Safinamide	3.482	616356	36589	5689.38	1.27
6	Safinamide	3.480	615847	36698	5698.49	1.30
Mean			615962			
Std. Dev			365.44			
%RSD			0.059			

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of Stock Solution:

Accurately weighed and transfered 10mg of Safinamide working standard into a 10ml clean dry volumetric flask added about 7ml of diluent and sonicated to dissolve it completely, volume was made up to the mark with the same solvent. (Stock solution)

Further pipetted 10 ml of the above stock solutions into a 100ml volumetric flask and diluted up to the mark with diluent. (100ppm of Safinamide)

Further pipette 1.0 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (10ppm of Safinamide)

Procedure:

The standard solutions prepared in the precision were injected on the other day, for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table-5: Results of Intermediate Precision for Safinamide

Sl. No.	Peak name	Retention time(min)	Peak Area	Height (µV)	USP Plate Count	USP Tailing
1	Safinamide	3.493	616865	36985	5748.96	1.30
2	Safinamide	3.491	616958	36859	5748.89	1.31
3	Safinamide	3.497	616352	36974	5748.28	1.29
4	Safinamide	3.491	616857	36989	5784.65	1.31
5	Safinamide	3.489	616539	36587	5846.27	1.30
6	Safinamide	3.489	616528	36969	5963.74	1.32
Mean			616683.2			
Std. Dev			242.22			
%RSD			0.039			

Specificity:

For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks.

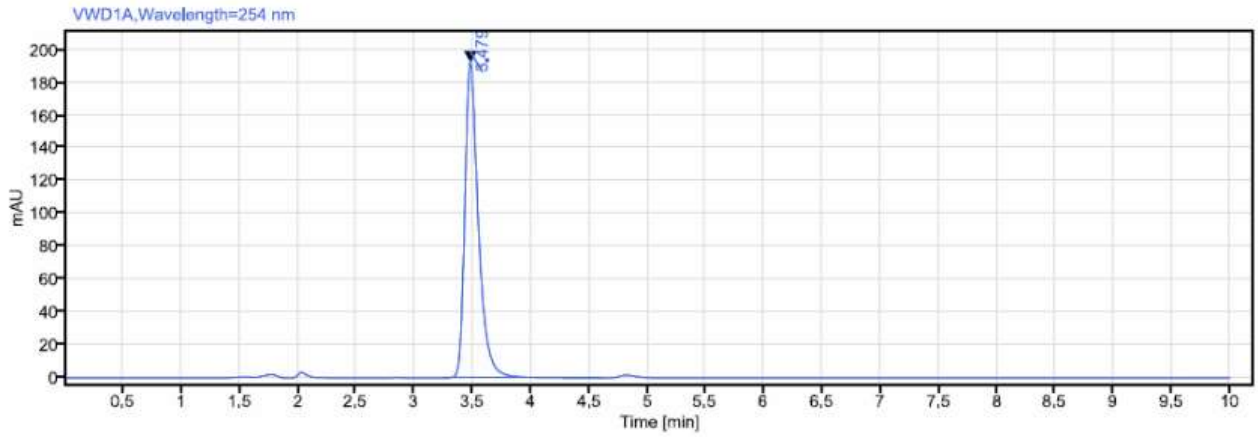

Fig-4: Standard Chromatogram for Specificity Standard

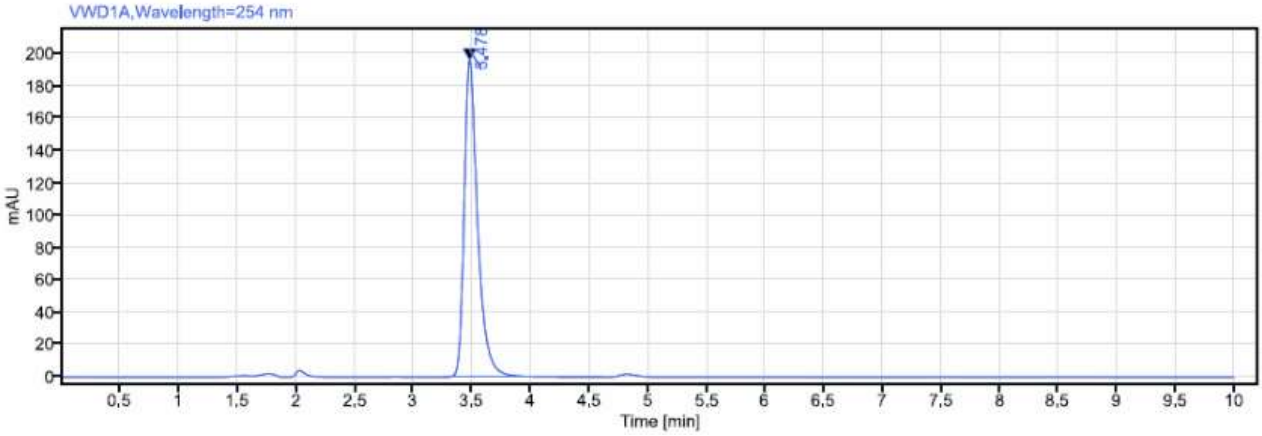

Fig-5: Sample Chromatogram for Specificity Sample

Accuracy:

Preparation of Standard Stock Solution:

Accurately weighed and transfered 10mg of Safinamide working standard into a 10ml clean dry volumetric flask added about 7ml of diluent and sonicated to dissolve it completely, volume was made up to the mark with the same solvent. (Stock solution)

Further pipetted 10 ml of the above stock solutions into a 100ml volumetric flask and diluted up to the mark with diluent. (100ppm of Safinamide)

Further pipetted 1.0 ml of the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent. (10ppm of Safinamide)

Preparation Sample Solutions:

For preparation of 80% solution (With respect to target Assay concentration):

Accurately weighed 10 tablets crushed in mortor and pestle and transfered equivalent to 10mg Safinamide (marketed formulation = 100mg of tablet Powder) into a 100ml clean dry volumetric flask added about 30 ml of diluent and sonicated it up to 30 mins to dissolve it completely, the volume was made up to the mark with the same solvent. Then it was filtered through 0.45 micron injection filter. (Stock solution)

Further pipetted 0.8ml of Safinamide from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (8ppm of Safinamide)

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weighed 10 tablets crushed in mortor and pestle and transfered equivalent to 10mg Safinamide (marketed formulation = 100mg of tablet Powder) into a 100ml clean dry volumetric flask added about 30 ml of diluent and sonicated it up to 30 mins to dissolve it completely, the volume was made up to the mark with the same solvent. Then it was filtered through 0.45 micron injection filter. (Stock solution)

Further pipetted 1.0 ml of Safinamide from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (10ppm of Safinamide)

For preparation of 120% solution (With respect to target Assay concentration):

Accurately weighed 10 tablets crushed in mortor and pestle and transfered equivalent to 10mg Safinamide (marketed formulation = 100mg of tablet Powder) into a 100ml clean dry volumetric flask added about 30 ml of diluent and sonicated it up to 30 mins to dissolve it completely, the volume was made up to the mark with the same solvent. Then it was filtered through 0.45 micron injection filter. (Stock solution)

Further pipetted 1.2 ml of Safinamide from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. (12ppm of Safinamide)

Procedure:

Injected the standard solution, Accuracy -80%, Accuracy -100% and Accuracy -120% solutions. Calculated the Amount found and Amount added for Safinamide and the individual recovery and mean recovery values were also calculated

Table-214: Results of Accuracy for Safinamide

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80%	8	7.99	584746	99.869	Mean = 99.87
S_2 : 80%	8	8.002	585634	100.022	S.D. = 0.14
S_3 : 80%	8	7.979	583968	99.734	% R.S.D. = 0.15
S_4 : 100%	10	9.944	725875	99.436	Mean = 99.89
S_5 : 100%	10	9.987	728976	99.865	S.D. = 0.47
S_6 : 100%	10	10.037	732658	100.375	% R.S.D. = 0.47
S_7 : 120%	12	12.03	876574	100.251	Mean = 100.34
S_8 : 120%	12	12.017	875657	100.145	S.D. = 0.24
S_9 : 120%	12	12.073	879686	100.61	% R.S.D. = 0.24

Linearity:

Preparation of Stock Solution:

Accurately weighed and transfered 10mg of Safinamide working standard into a 10ml clean dry volumetric flask added about 7ml of diluent and sonicated to dissolve it completely, volume was made up to the mark with the same solvent. (Stock solution)

Further pipetted 10 ml of the above stock solutions into a 100ml volumetric flask and diluted up to the mark with diluent. (100ppm of Safinamide)

Preparation of Level – I (6 ppm of Safinamide):

0.6 ml of above stock solutions was taken in different 10ml of volumetric flasks, diluted up to the mark with diluent.

Preparation of Level – II (8 ppm of Safinamide):

0.8 ml of above stock solutions was taken in different 10ml of volumetric flasks, diluted up to the mark with diluent.

Preparation of Level – III (10 ppm of Safinamide):

1.0 ml of above stock solutions was taken in different 10ml of volumetric flasks, diluted up to the mark with diluent.

Preparation of Level – IV (12 ppm of Safinamide):

1.2 ml of above stock solutions was taken in different 10ml of volumetric flasks, diluted up to the mark with diluent

Preparation of Level – V (14ppm of Safinamide)

1.4 ml of above stock solutions was taken in different 10ml of volumetric flasks, diluted up to the mark with diluent

Procedure:

Each level was injected into the chromatographic system and measured the peak area. A graph was plotted of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculated the correlation coefficient.

Table-7: Linearity Results: (for Safinamide)

S. No	Linearity Level	Concentration	Area
1	I	6	428654
2	II	8	565865
3	III	10	715875
4	IV	12	856687
5	V	14	1005473

Correlation Coefficient	0.9998

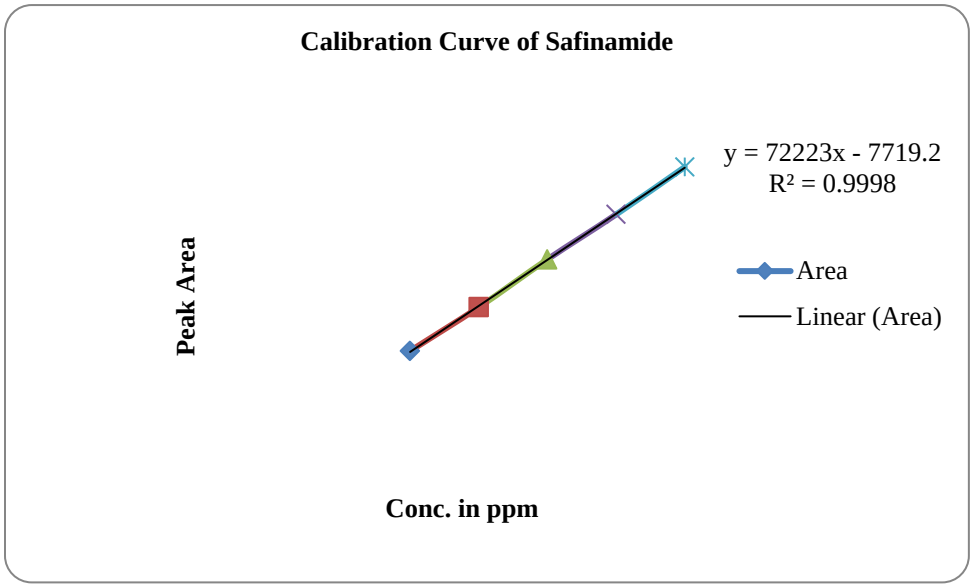

Fig-6: Calibration Curve of Safinamide

Acceptance Criteria: Correlation coefficient should be not less than 0.99.

Limit of Detection and Limit of Quantitation:

LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Observation: On the evaluation of above results the LOD and LOQ for the Safinamide was found to be 0.6030 µg/ml and 1.8273µg/ml respectively.

Robustness:

As part of the Robustness, deliberate change in the Flow rate and Mobile Phase composition was made to evaluate the impact on the method.

A. The flow rate was varied at 0.18 ml/min to 0.22 ml/min.

Standard solution 10ppm of Safinamide was prepared and analysed using the varied flow rates along with method flow rate.

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

Table-8: System suitability results for Safinamide

Sl. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.18	5785.38	1.36
2	0.2	5685.76	1.28
3	0.22	5748.92	1.37

***** Results for actual flow (0.2 ml/min) have been considered from Assay standard.

B. The Changes in the Detection Wavelength was varied from ±5.

Standard solution 10µg/ml of Safinamide was prepared and analysed using the varied Changes in the Detection Wavelength along with the actual Wavelength in the method.

On evaluation of the above results, it can be concluded that the variation in the Detection Wavelength has not affected the method significantly, indicating that the method is robust even by change in the Detection Wavelength ±5.

Table-9: System suitability results for Safinamide

Sl. No.	Change in Wavelength	System Suitability Results	
		USP Plate Count	USP Tailing
1	-5	5865.36	1.31
2	*Actual	5685.76	1.28
3	+5	5748.95	1.36

***** Results for actual wavelength Detection 254nm has been considered from Accuracy standard

Assay of Marketed Formulation:

Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, the volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. Further dilution of the above solution was made by taking 1ml from it and diluting up to 10 ml with the diluent to give 10 ppm solution. The solution was filtered through a membrane filter (0.45 ⌈m) and sonicated to degas. The solution prepared was injected in three replicates into the UPLC system and the observations were recorded.

Calculation: (For Safinamide)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT = Average area counts of sample preparation.

AS = Average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim mg/ml.

Result & Discussion: The %Purity[29] of Marketed Formulation of Safinamide was found to be 100.57%.

Degradation Studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Safinamide using the proposed method.

Preparation of Stock:

Accurately weighed 10 tablets crushed in mortor and pestle and transferred equivalent to 10mg Safinamide (marketed formulation= 100mg of tablet Powder) sample into a 100ml clean dry volumetric flask added about 30 ml of diluent and sonicate it up to 30 mins to dissolve it completely, the volume was made up to the mark with the same solvent. Then it was filtered through 0.45 micron injection filter. (Stock solution)

1. **Hydrolytic Degradation under Acidic Condition**

   Pipetted 1.0 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept for 24 hours and then neutralized with 0.1 N NaOH and made up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and placed in vials.

2. **Hydrolytic Degradation under Alkaline Condition**

   Pipetted 1.0ml of above solution into a 10ml volumetric flask into a 10ml volumetric flask and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept for 24hours and then neutralized with 0.1N HCl and made up to 10ml with diluent. Filtered the solution with 0.45 microns syringe filters and placed in vials.

3. **Oxidative Degradation**

   Pipetted 1.0ml above stock solution into a 10ml volumetric flask solution into a 10ml volumetric flask 1 ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask

and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 24 hours. Filtered the solution with 0.45 microns syringe filters and placed in vials.

4. **Thermal Degradation**

   Safinamide sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents to 10ppm and injected into UPLC and analysed.

5. **Photolytic Degradation:** Safinamide sample was taken in petridish and exposed to sunlight for 24hrs. Then the sample was taken and diluted to 10ppm with diluent, filtered the solution with 0.45 micron syringe, injected into UPLC and analysed.

Table-10: Results of Degradation Studies

Sample Name	Safinamide	
	Area	% Degraded
Standard	615875	0.00
Acid	183998.815	29.876
Base	193908.243	31.485
Peroxide	42470.74	6.896
Thermal	165331.643	26.845
Photo	128526.953	20.869

SUMMARY AND CONCLUSION

In conclusion, a simple, accurate, sensitive, rapid, and precise UPLC method was developed and validated for the estimation of Safinamide in the bulk form and marketed pharmaceutical dosage form. Statistical analysis for the above said results demonstrates that the method is fit for the estimation of Safinamide in bulk and pharmaceutical forms. The assay values were in good agreement with their respective labeled claim. The absence of interfering peaks in the chromatogram suggests that the tablet excipients do not interfere with the estimation of the drug by the proposed method. Hence, it is concluded that the proposed method can be utilized for

research studies, quality control, and routine analysis for the quantification of Safinamide in tablet dosage form with lesser resources available.

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