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QbD Based Rp-Hplc Method Development And Validation for The Estimation Of Safinamide In Bulk And It's Dosageform

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ABSTRACT

A simple and selective HPLC method is described for the determination of Safinamide by QbDChromatographic separation was achieved on a Zorbax C18 (150mm×.4.6mm & 5µm) usingmobile phase consisting of a mixture of pH 2.3 Buffer: Acetonitrile (64:34) with detection of226nm Linearity was observed in the range 50-150 µg /ml for Safinamide (r^2 =0.9997) for drugsestimated by the proposed methods was in good agreement with.3 the label claim. Thus, The RP-HPLC assay method developed for Safinamide by QbD approach is linear, accurate, precise,reproducible, and specific as evident from the validation results. The developed method is alsostability indicating and can be conveniently used for quality control to determine the assay inregular Safinamide product development, production, and stability samples.A new ReversePhase-HPLC method was developed for safinamide using Design Expert 9 software. In thissoftware, 3^2 factorial statistical designs were used to optimize the Critical Process Parameters orCritical Method Parameters and to evaluate interaction effects of these parameters on the CriticalQualityAttributes.

Keywords: Safinamide,HPLC,QbD,Designexpert,3^2 factorialstatisticaldesign.

INTRODUCTION

Safinamide is a selective and reversible MAO-B inhibitor. The mechanism of action is unknown,but it is thought that blocking the catabolism of dopamine via MAO-B inhibition increasesextracellularlevelsofdopamineinthestriatumandsubsequentlyincreasesdopaminergicactivity.
Safinamideisindicatedasanadd-ontherapytoaregimenthatincludeslevodopaforthetreatment of the signs and symptoms of idiopathic Parkinson disease in patients experiencingOFF episodes while on a stable dose of levodopa. Safinamide has not been shown to be effectiveas monotherapy for the treatment of Parkinson disease. Safinamide is available as 50 mg and 100mgtablets (as safinamidemesylate) for oral use.
The recommended starting dose for safinamide is 50 mg once per day, administered orally. Aftertwo weeks, the dose may be increased to 100 mg once per day based on individual clinical needand tolerability. When discontinuing treatment, safinamide 100 mg/day should be tapered bydecreasingthe doseto 50mg/dayfor oneweek prior to stopping.

Structure

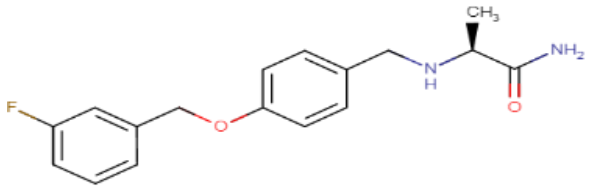


Fig 1: Structure of safinamide

IUPACNAME: (2S)-2-[({4-[(3-fluorophenyl)methoxy]phenyl} methyl) amino]propanamide

MATERIALS AND METHODS

Table 1: Instruments

Instruments	Model
UV-Visible Spectrophotometer	Thermo Electron corporation
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Open LabEZ Chrome
HPLC	SHIMATZO1200
Ultrasonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Thermo scientific
Electronic balance	Mettler Toledo
Column	Waters Acquity C18 (150x2.2mmID4.6µm)

Table 2: Reagents

Name of the solvent	Solubility results
Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogenortho phosphate	AR Grade
Acetonitrile	HPLC Grade
Disodium hydrogen phosphate	AR Grade

Determination of working wavelength (λmax)

Preparation of Standard Solution

10 mg of Safinamide was weighed and transferred in to 100 ml volumetric flask and dissolved inmethanol and then make up to the mark with methanol and prepare 10 µg /ml of solution bydiluting1ml to 10ml with methanol.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ max) of the solution of the drug in mobile phase were scanned using UV-Visible spectrophotometer within the wave length region of 200–400nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at264 nm for Safinamide (Fig.1.1), 226 nm was selected as detector wavelength for the HPLC chromatographic method.

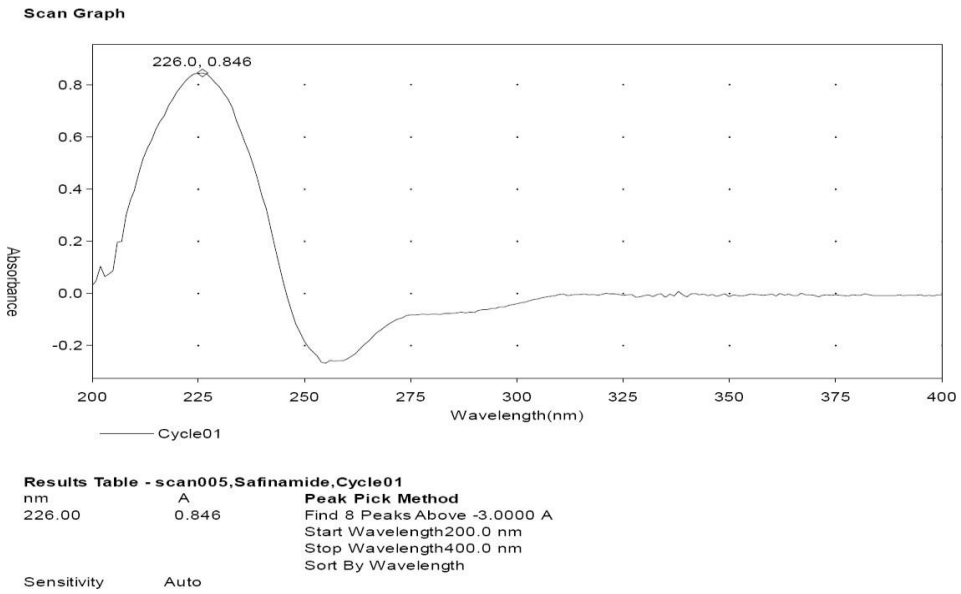


Fig 2: UV-VIS Spectrum of Safinamide (226nm)

Safinamide assay optimization by 3^2square factorial design

Initial method development

Zorbax C18 (150mm×.4.6mm&5µm) was selected for Assay optimization of Safinamide.

Software aided method development

A new Reverse Phase-HPLC method was developed for the determination of Safinamide by using QbD approach. A Quality by Design with Design of Experiments approach to the development of an analytical method mainly involves two phases as follows:

a) Screening Phase
b) Statistical Analysis and
c) Final Optimization

ScreeningPhase

A new Reverse Phase-HPLC method was developed for safinamide using Design Expert 9 software. In this software, 3^2 factorial statistical designs were used to optimize the Critical Process Parameters (CPP) or Critical Method Parameters (CMPs) and to evaluate interaction effects of these parameters on the Critical Quality Attributes (CQAs).

Selection of Critical Method Parameters

Critical Method Parameters are selected of number of factors that impact on the analytical technique under development. So, the Critical Method Parameters selected for the study are Buffer pH, Organic Phase (%acetonitrile) and Organic Modifier (Acetonitrile).

Selection of Critical Quality Attributes (CQAs)

Critical Quality Attributes are the responses that are measured to judge the quality of the developed analytical methods. So, the Critical Quality Attributes selected for the study areRetention time and Tailing Factor. These responses were monitored during the experimental trials.

Statistical Analysis and Final Optimization

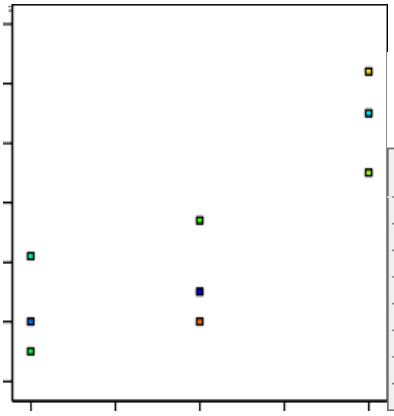
The responses obtained after carrying out the above trial runs were fed back to Design Expertsoftware and plots like contour plots and Graph plots were plotted. These plots revealed theinfluence of critical method parameters on the selected quality attributes. The analysis of these plots was used to estimate as to which method parameter gave the most acceptable responses.

Table 3: Optimization of parameters for analysis of Safinamide using Design Expert software

	Factor1	**Factor2**	**Response1**	**Response2**
Run	A:pH	B:organicratio	R.T	Tailingfactor
1	2.50	30.00	3.5	1.5
2	2.30	30.00	3	1.2
3	2.70	30.00	6.5	1.9
4	2.30	20.00	4.1	1.5
5	2.30	40.00	2.5	1.1
6	2.50	20.00	4.7	1.7
7	2.70	40.00	5.5	2
8	2.70	20.00	7.2	2.2
9	2.50	40.00	3	1.4

Graphplots

Correlation: 0.836 Color points by Run



ANOVA for Quadratic model

Response 1: R.T

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	21.86	5	4.37	128.66	0.0011	significant
A-pH	15.36	1	15.36	452.01	0.0002	
B-organic ratio	4.17	1	4.17	122.62	0.0016	
AB	0.0025	1	0.0025	0.0736	0.8038	
A^2	2.28	1	2.28	66.96	0.0038	
B^2	0.0556	1	0.0556	1.63	0.2910	
Residual	0.1019	3	0.0340			
Cor Total	21.96	8				

AdjustedR^2 Equation

Std. Dev.	0.1843	R^2	0.9954
Mean	4.44	Adjusted R^2	0.9876
C.V. %	4.15	Predicted R^2	0.9507
		Adeq Precision	32.3338

R.T	=
+3.62	
+1.60	* A
-0.8333	* B
-0.0250	* AB
+1.07	* A^2
+0.1667	* B^2

Response 1: R.T

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0014		0.8521	0.7774	
2FI	0.9456		0.8227	0.5730	
Quadratic	**0.0086**		**0.9876**	**0.9507**	**Suggested**
Cubic	0.5742		0.9878	0.7211	Aliased

FactorCoding:Actual

R.T

DesignPoints

40.00

R.T

2.5

X1 = AX2=B

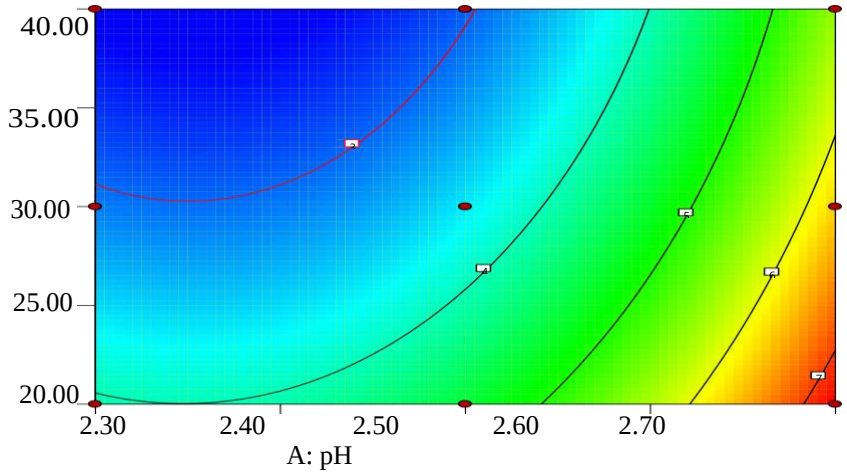


Fig 3: Diagramof annova quadratic model for retention time

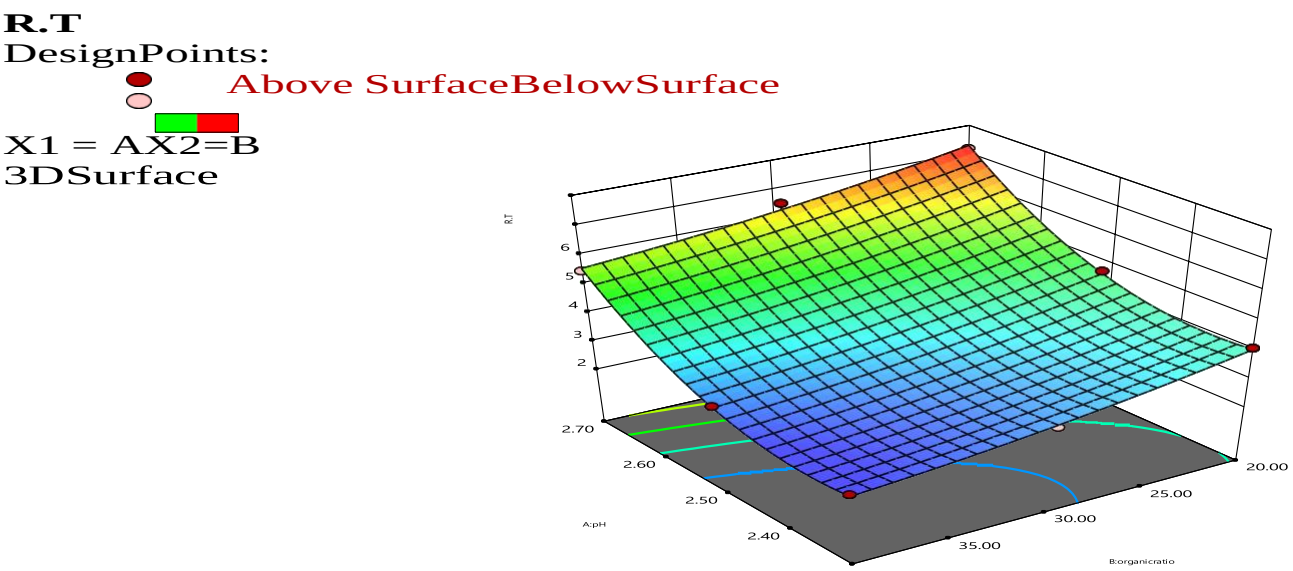


Fig 4: Three-dimensional plot for retention time for pH and organic ratio

Tailing factor

Response 2: Tailing factor

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0003		0.9116	0.8283	
2FI	0.4111		0.9086	0.6336	
Quadratic	**0.0442**		**0.9810**	**0.9323**	**Suggested**
Cubic	0.7559		0.9673	0.2561	Aliased

Response 2: Tailing factor

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.08	5	0.2162	83.40	0.0020	significant
A-pH	0.8817	1	0.8817	340.07	0.0003	
B-organic ratio	0.1350	1	0.1350	52.07	0.0055	
AB	0.0100	1	0.0100	3.86	0.1443	
A^2	0.0272	1	0.0272	10.50	0.0478	
B^2	0.0272	1	0.0272	10.50	0.0478	
Residual	0.0078	3	0.0026			
Cor Total	1.09	8				

Adjusted R^2 Equation

Std. Dev.	0.0509		R^2	0.9929
Mean	1.61		**Adjusted R^2**	0.9810
C.V. %	3.16		**Predicted R^2**	0.9323
			Adeq Precision	25.6571

Tailing factor	=
+1.46	
+0.3833	* A
-0.1500	* B
+0.0500	* AB
+0.1167	* A^2
+0.1167	* B^2

Tailing factor
DesignPoints
Tailing factor

1.1 2.2

X1 = AX2=B

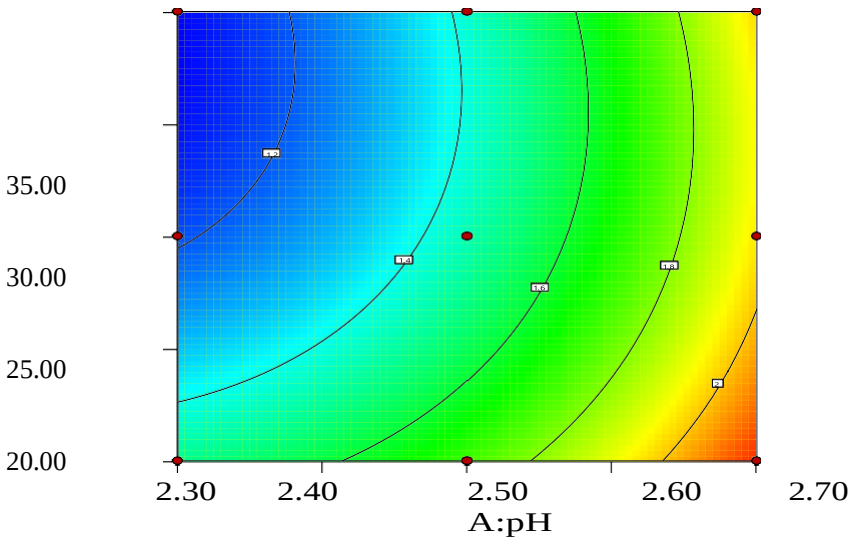


Fig 5: Diagramof annova quadratic model for tailing factor

Factor coding: Actual

Tailing factor
Designpoints:
Above surfacebelowsurface

1.1 2.2

X1 = AX2=B
3dsurface

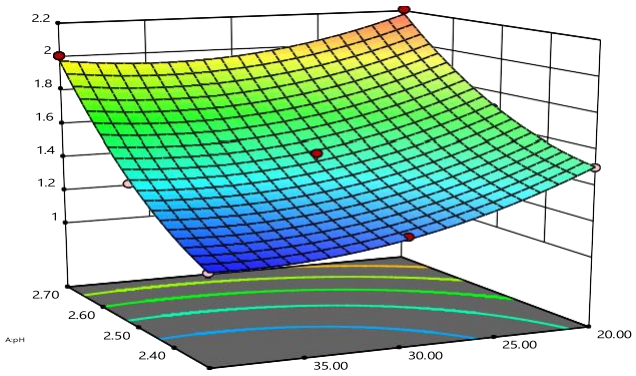


Fig 6: Three-dimensionalplotfortailingfactorforphandorganicratio

Desirability

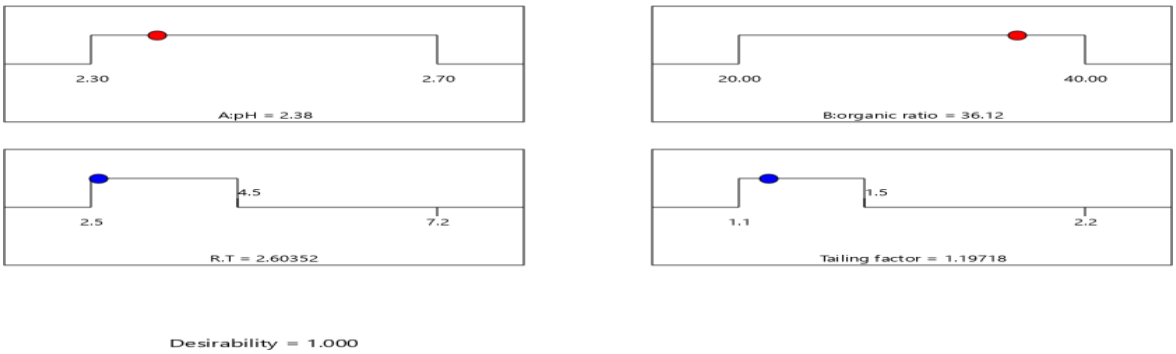


Fig 7: Diagram of Desirability Value

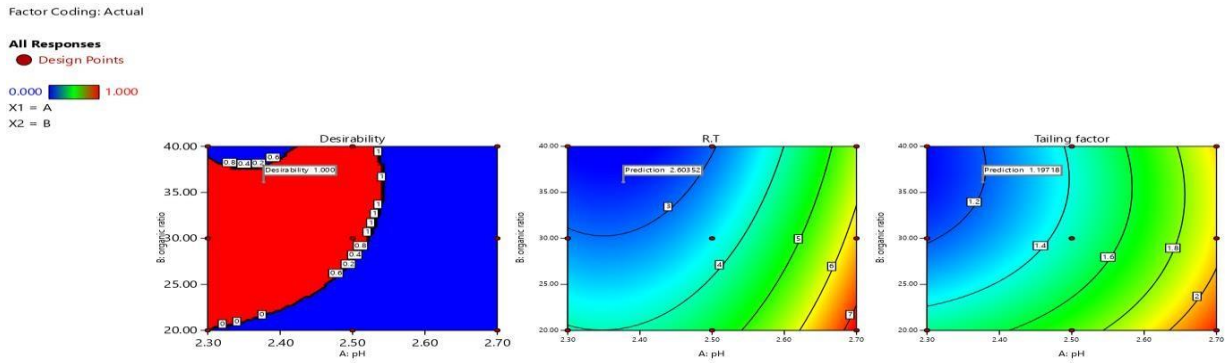


Fig 8: 3D Diagram of Desirability, Retention time and Tailing factor

Table 4: Final optimization results

Critical method parameters	Low level	MediumLevel	High level	Final level Selected
BufferpH	2.3	2.5	2.7	2.3
Organicratio	20	30	40	40

Optimized conditions

Column : Zorbax C18(150mm×.4.6mm&5µm)
Mobile phase: pH2.3Buffer :Acetonitrile
Ratio: 64:34
Column Oven Temperature: 25°C
Flow rate: 1.00mL/min
Retention time: 2.535
Detection wave length: 226 nm
Injection volume: 10µL
Run time: 10 min

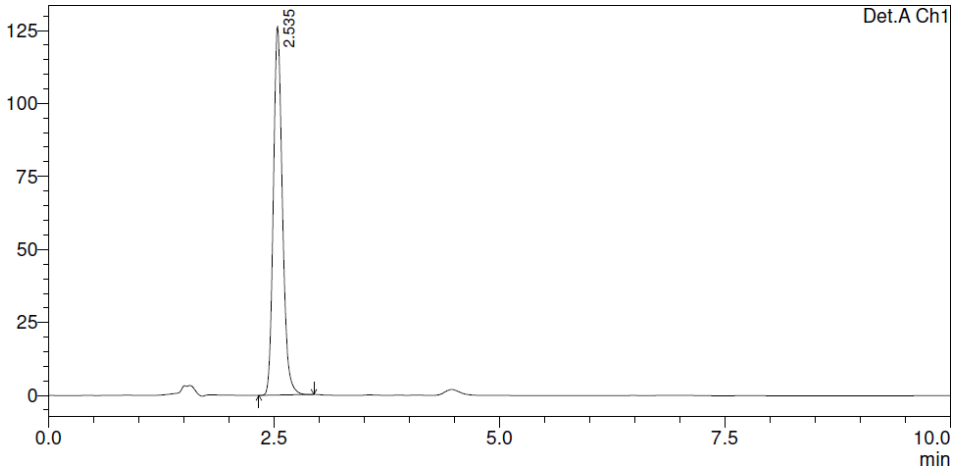


Fig 9: OptimizedChromatogramof Safinamide

Method validation parameters

- System Suitability and system Precision
- Specificity (Blank and Placebo Interference)
- Method Precision–Repeatability
- Intermediate precision-Reproducibility (Rugged)ness
- Linearity and range
- Accuracy and recovery
- Limit of detection
- Limit of quantification
- Robustness

System suitability and system precision preparation of the Standard

Weighed accurately 100mg Safinamide in 100 ml of volumetric flask and dissolve in70ml of mobile phase and make up the volume with mobile phase from above stocksolution 100µg/ml of Safinamide was prepared by diluting 5ml to 50ml with mobile phase respectively.

System suitability and System Precision Acceptance Criteria

- The % RSD of the area response of Standard peak

obtained from the six injections of standard solution should beno more than 2.0

- The Theoretical plates for 1st injection should be NLT2000 for both Safinamide peak.
- The Tailing factor for 1st injection should be NMT2.0 for Safinamide peak

Table 5: System suitabilityresults

Name of the Standard	Area of Safi amide	Tailing factor	Plate count
Standard-01	885254	1.24	18412
Standard-02	884510	1.24	18444
Standard-03	885607	1.24	18493
Standard-04	886616	1.25	18428
Standard-05	885223	1.24	18518
Average	885442	1.24	18459
%RSD	0.1	0.4	0.2

Observation

System suitability results were met with accept an ceriteria, hencesystem issuitable

Table 6: System Precisionresults

Name of the Standard	Area of Safinamide
Standard-01	885254
Standard-02	884510
Standard-03	885607
Standard-04	886616
Standard-05	885223
Standard-06	889600
Average	886135
%RSD	0.2

System Precision results met with acceptance criteria hence the system is precise.

Specificity

Blank and Placebo Interference

Preparation of the Standard: Weighed accurately 100mg Safinamide in 100 ml of volumetric flask and dissolve in 70ml of mobile phase and make up the volume with mobile phase From above stock solution 100µg/ml of Safinamide was prepared by diluting 5ml to 50ml with mobile phase respectively.

Preparation of sample solution: 20tablets (each tablet contains 50mg of Safinamide) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weighedcrushed powder equivalent to 100mg of Safinamide in 100 ml of volumetric flask and dissolve in70ml of mobile phase by 30min of sonication and make up the volume with mobile phase.Centri fuged sample at 5000rpm for 10min.

From above stock solution 100µg/ml of Safinamide is prepared by diluting 5ml to 50ml withmobilephaserespectively.

Preparation of Placebo solution: Weigh Placebo powder equivalent to 100mg of Safinamide in 100 ml of volumetric flask anddissolve in 70ml of mobile phase by 30min of sonication and make up the volume with mobilephase. Centrifuged sample at 5000rpm for 10min. Diluted 5ml to 50ml with mobilephaserespectively.

No interference should be observed at the retention time of Safinamide due to blank andPlacebo.

Table 7: Results of Specificity

S.No.	Solution details	Area of Safinamide
1	Standard	1061253
2	Blank	Not Detected
3	Placebo solution	Not Detected
4	Test solution	1059702

There was no interference observed at the retention time of Safinamide due to blank and Placebo Hence System is specific

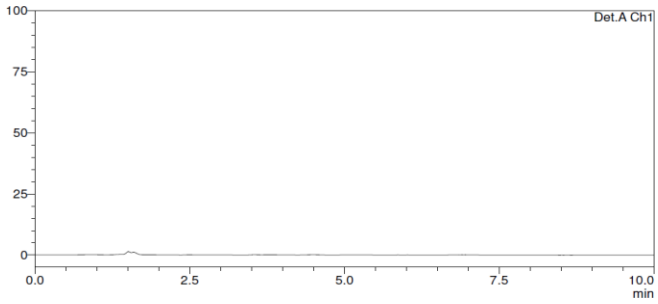


Fig 10: Chromatogram of Blank

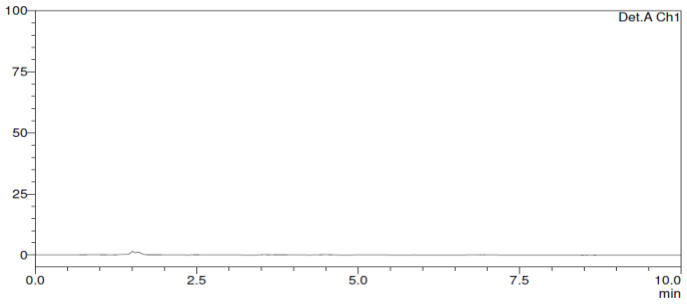
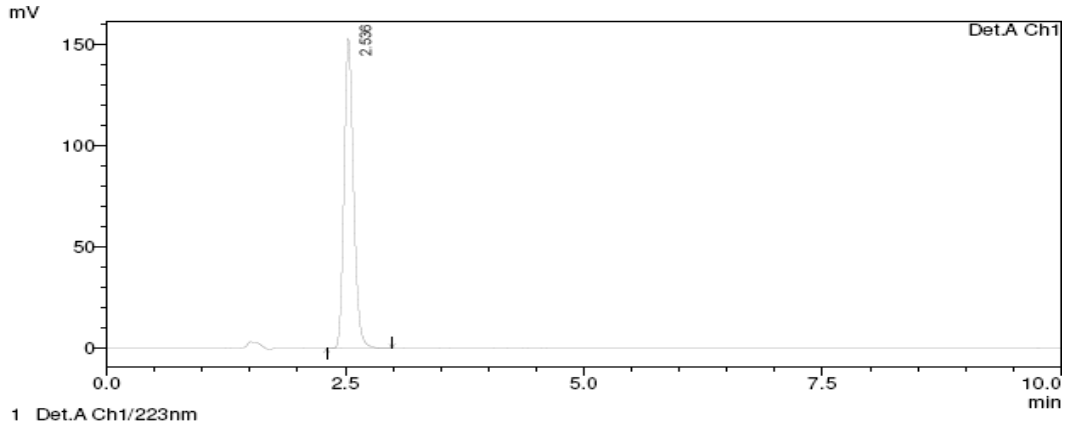


Fig 11: Chromatogram of Placebo

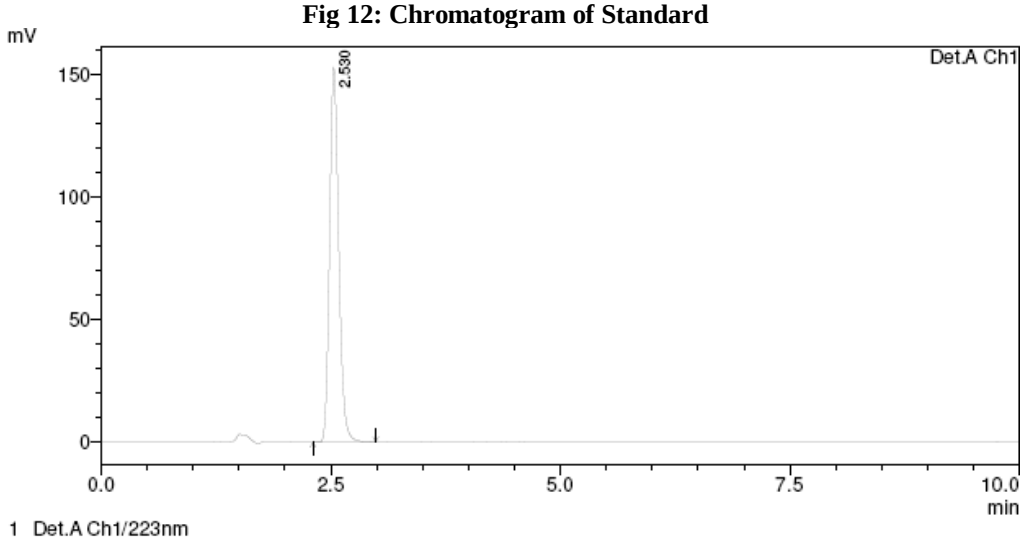


PeakTable

tector A Ch1 223nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met	Area %	Tailing Factor
1	2.536	1061253	154023	18650.223	100.000	1.253
Total		1061253	154023		100.000	

Fig 12: Chromatogram of Standard



PeakTable

tector A Ch1 223nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met	Area %	Tailing Factor
1	2.530	1059702	153085	18706.080	100.000	1.251
Total		1059702	153085		100.000	

Fig 13: Chromatogram of sample

Precision

Method precision

Preparation of the Standard: Weighed accurately 100mg Safinamide in 100 ml of volumetric flask and dissolve in 70ml of mobile phase and make up the volume with mobile phase from above stock solution 100µg/ml of Safinamide was prepared by diluting5ml to 50ml with mobile phase respectively.

Preparation of sample solution: 20tablets (each tablet contains 50mg of Safinamide) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weighed

crushed powder equivalent to 100mg of Safinamide in 100 ml of volumetric flask and dissolved in 70ml of mobile phase by 30min ofsonication and make up the volume with mobile phase. Centrifuged sample at 5000rpm for10min. From above stock solution 100µg/ml of Safinamide is prepared by diluting 5ml to 50ml with mobile phase respectively Prepared another five-sample preparation in above manner

- Mean %Assay should be 90.0 to 110.0%for both Safinamide
- The %RSD of %Assay results obtained from Test solution should not be more than 2.0% for Safinamide

Table 8: Method Precision Results

S.No.	Solution details	%Assay of Safinamide
1	Test solution preparation 1	101.4
2	Test solution preparation 2	101.2
3	Test solution preparation 3	101.1
4	Test solution preparation 4	101.2
5	Test solution preparation 5	100.9
6	Test solution preparation 6	101.4
	Average	101.2
	StdDev	0.21
	%RSD	0.20

- Mean %Assay Obtained between90.0to 110.0% forSafinamide
- The %RSD of %Assay results obtained from Test solution was obtained less than 2.0% for Safinamide
- Hence Method is Precise

Intermediate precision

- Mean %Assay should be 90.0 to 110.0% for Safinamide (Performed byAnalyst-II)
- The %RSD of %Assay results obtained from Test solution should not be more than 2.0% for Safinamide (Performed by Analyst-II)
- Cumulative %RSD of %Assay results obtained from both analysts-I&II test solution should not be more than 2.0%for both Safinamide
- Cumulative Mean of %Assay for both analysts-I &II should be 90.0 to 110.0% for Safinamide

Table 9: Intermediate Precision Results

S.No.	Solution details	%Assay of Safinamide
1	Solution-1	99.8
2	Solution-2	99.8
3	Solution-3	100.0
4	Solution-4	100.2
5	Solution-5	100.1
6	Solution-6	99.9
	Average	**100.0**
	StdDev	**0.21**
	%RSD	**0.2**

Mean %Assay obtained between 90.0 to 110.0% for Safinamide

- The %RSD of %Assay results was obtained from Test solution less than 2.0% for Safinamide (Performed by Analyst-II)
- Cumulative %RSD of %Assay results was obtained less than 2.0 for both an alysts-I&II of Safinamide
- Cumulative Mean of %Assay for both analysts-I &II obtained 90.0 to 110.0% for Safinamide
- Hence Method is Rugged

Linearity and range

Preparation of the Standard Stock: Weigh accurately 50mg Safinamidein 100ml of volumetric flask and dissolve in 70ml of mobile phase and makeup the volume with mobile phase and mix well.

Table 10: Linearity & Range of Safinamide

Volume Taken (mL)	Volume diluted to	Concentration (µg/mL)
5	200	25
5	100	50
4	50	80
5	50	100

3	25	120
3	22	150

The correlation coefficient value should not be less than0.99 for Safinamide

Table 11: Linearity Results

S.No	Name of the Solution	Area of Safinamide
1	Level-1(25%)	204521
2	Level-2(50%)	408150
3	Level-3(80%)	659552
4	Level-4(100%)	816089
5	Level-5(120%)	967382
6	Level-6(150%)	1200219
Slope		8024.6
Intercept		6145.3
Correlation coefficient		0.9997
LOQ in µg/mL		9.96
LOD in µg/mL		3.03

Fig 14: Calibration curve of Safinamide

The correlation coefficient value obtained 0.9997 for Safinamide

Accuracy and recovery

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzedsample), the reference standards of the drugs were added at the level of 50%, 100%& 150%.The recovery studies were carried out three times and the percentage recovery and percentagemean recovery were calculated for drug is shown in table. To check the accuracy of the method,recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%&150%.

- The %Recovery should be 98.0 to 102.0% for Safinamide
- Mean %Recovery should be 98.0 to 102.0% for Safinamide
- %RSD for All % recoveries should be NMT2.0%

Table 12: Results of Accuracy

Recovery level	Accuracy Safinamide			Average % Recovery
	Area	Average area	%Recovery	
50%	445655	446122	100.7	99.8 %
	446127			
	446584			
100%	876186	876565	99.0	
	876454			
	877056			
150%	1327020	1325898	99.8	
	1324388			
	1326287			

- The %Recovery obtained between 98.0 to 102.0%forSafinamide
- Mean %Recovery obtained between 98.0 to102.0% for Safinamide
- %RSD obtained for All %recoveries less than 2.0%
- Hence method is Accurate

Limit of detection

LOD = 3.3σ S
LOD =3.3 * (767.70)/ 8024.6
LOD =3.03µg/ml
Where,σ = the standard deviation of the responseS=the slopeofthe calibration curve
Slope S may be estimated from the calibration curve of the analyte.
The LOD forth is method was found to be3.03µg/ml

Limit of quantification

LOQ =10σ S
LOQ =10 * (767.70)/8024.6
LOQ =9.96µg/ml
Where, σ = the standard deviation of the response S = the slope of the calibration curve
Slope S may be estimated from the calibration curve of the analyte.
The LOD for this method was found to be9.96µg/ml

Robustness

- The %RSD of the area response of Safinamide peak obtained from the five injections of standard solution should beno more than 2.0
- The Theoretical plates for 1st injection should be NLT1500 for Safinamide peak.
- The Tailing factor for 1st injection should be NMT2.0 for Safinamide peak

Table 13: Results of Robustness

Name of the Parameter	%RSD	Theoretical Plates	Tailing factor
Low Column Oven Temperature (20°C)	0.57	16252	1.31
High Column Oven Temperature (30°C)	0.53	17854	1.34
Lower Wave length (224nm)	0.14	16258	1.36
Higher Wave length (228nm)	0.25	14263	1.33

System suitability me the acceptance criteria in Robustness parameters hence method is Robust.

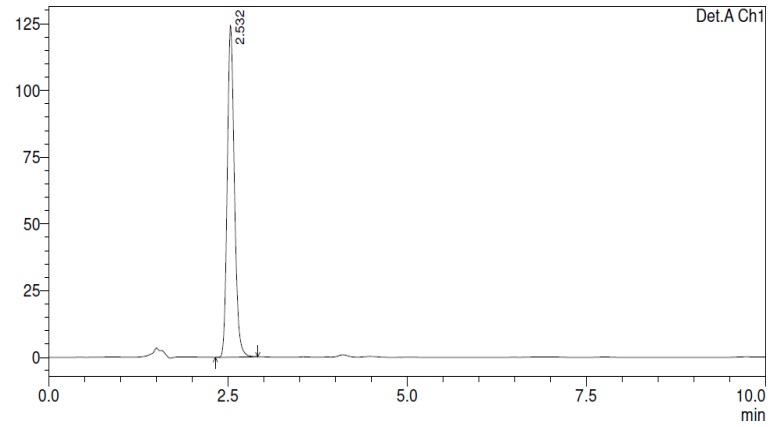


Fig 15: Chromatogram of Low Column Oven Temp (20°C)

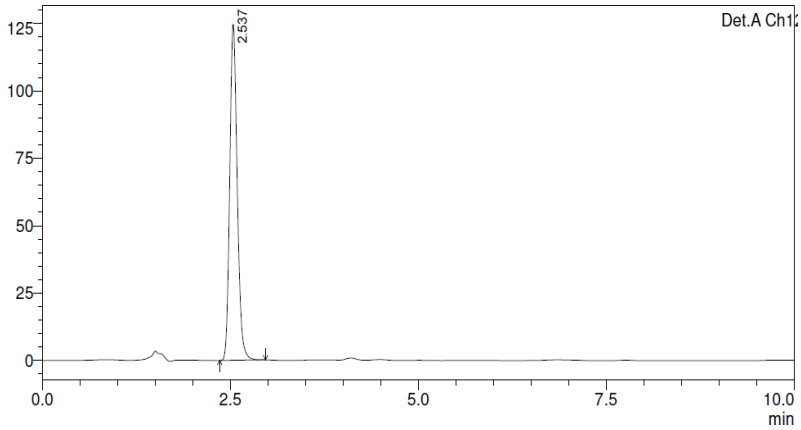


Fig 16: Chromatogram of Low Column Oven Temp (30°C)

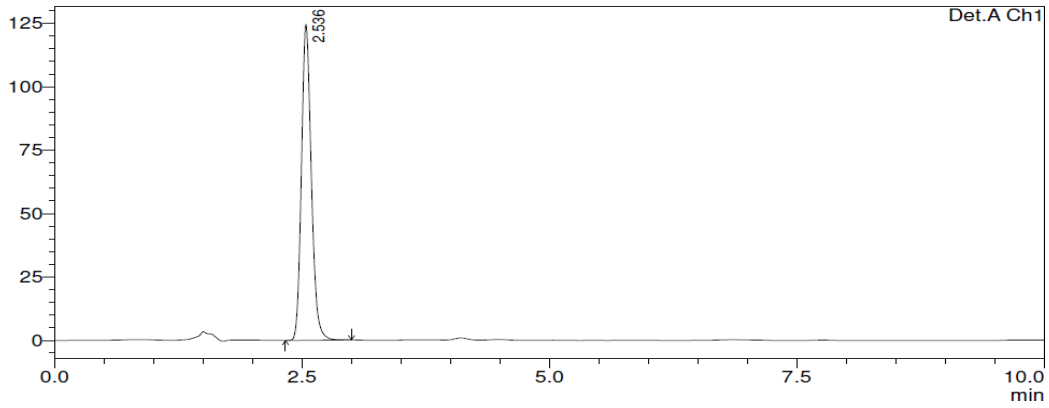


Fig 17: Chromatogram of Low Wave length (224nm)

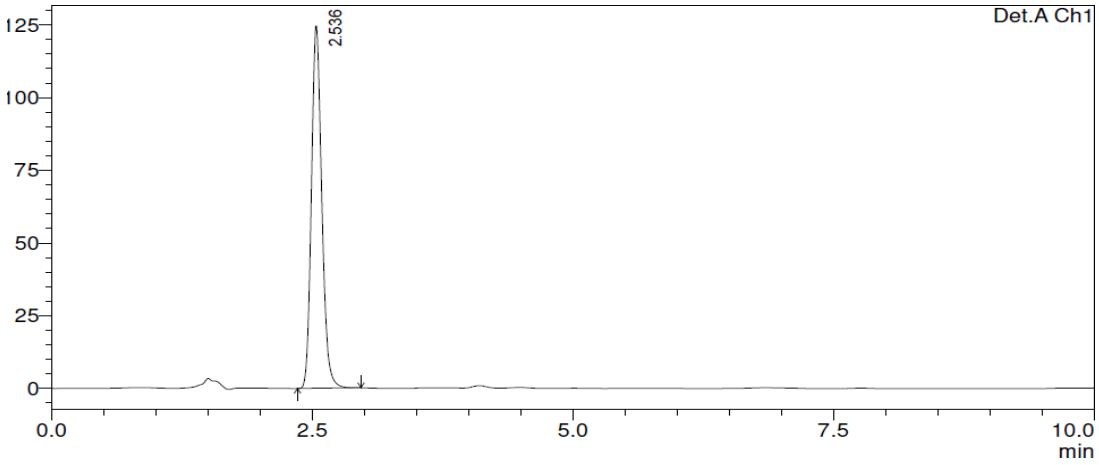


Fig 18 : Chromatogram of Higher Wave length (228nm)

CONCLUSION

Thus,The RP-HPLC assay method developed for Safinamide by QbD approach is linear, accurate, precise, reproducible, and specific as evident from the validation results. The developed method is also stability indicating and can be conveniently used for quality control to determine the assay in regular Safinamide product development, production, and stabilitysamples.A simple analytical and robust HPLC method was developed for the determination of Safinamide by using QbD approach using Design Expert® software. Results which were obtained from the validation of the developed analytical method were within the limit as per ICH guidelines.

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