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Analytical Method Development And Validation Of Rp-Hplc Method For Estimation Of Safinamide In Bulk And Pharmaceutical Dosage Form.

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ABSTRACT

Background: A rapid, highly sensitive, high-performance liquid chromatographic method has been developed to determine safinamide in bulk drug and pharmaceutical dosage form. The separation was performed using an HPLC method with a UV Openlab EZ Chrome workstation program, as well as a Kromasil 100 C18 (125 mm X 4.0 mm i.d.) 5 µm. 0.2 % OPA and methanol (65:35 v/v) were pumped at a flow rate of 1.0 mL/min and detected at 226 nm.

Result: The developed RP-HPLC method yielded a suitable retention time for safinamide of about 3.7 minutes, which was optimized on a trial-and-error basis. The linear response correlation coefficient (r^2 = 1.0000) was observed in the range of 20- 60µg/ml. The percentage RSD for the method's precision was found to be less than 2.0 percent. Validation parameters such as specificity, accuracy, linearity, precision, and robustness were also determined.

Conclusion: The developed and validated RP-HPLC system can be used in the industry for routine quality control/analysis of bulk drug and marketed safinamide products..

Keywords: *Safinamide, Development and validation, RP-HPLC, Accuracy, Precision.*

1. INTRODUCTION

Parkinson's disease (PD) is the second most prevalent chronic progressive neurological disorder in older adults, after Alzheimer's disease [1–3]. During "off" episodes of Parkinson's disease, safinamide is used as an adjuvant treatment. One of its several mechanisms of action is the inhibition of monoamine oxidase B [4].

Under the brand names Xadago and Xafinact in India, it is sold all over the world. According to a review of the literature, not much research has been done on the anti-Parkinsonian Medication Safinamide. It was discovered that the HPTLC method was frequently employed, but not the HPLC method [5].

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

Molecular Formula: C17H19FN2O2

Molecular Weight: 302.34 g/mol

Solubility: It is sparingly soluble in ethanol and is practically insoluble in ethyl acetate.

pH: Highly soluble at pH 1.2 and 4.5.

pKa: 7.93

Bioavailability: 95%

Drug approval: by USFDA in March 2017 and in India by CDSCO in 2019.

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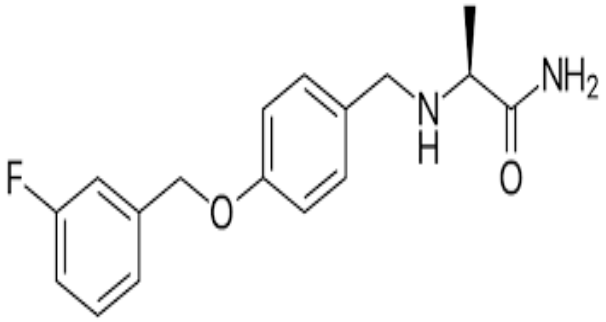

Fig. 1: Molecular Structure of Safinamide

Mechanism of action:

A special chemical, safinamide has a high therapeutic index and several modes of action. It blocks voltage-dependent Na+ and Ca2+ channels, inhibits glutamate release, and inhibits MAO-B in a powerful, selective, and reversible manner.

Absorption

Total bioavailability is 95%, and peak plasma concentrations occur between 2 and 4 hours. Food accelerated the pace of safinamide absorption while having no influence on its intensity.

Metabolism

The primary step is mediated by amidases which have not been identified, and produces safinamide acid. Moreover, it is converted to N-delkylated amine and O-debenzylated safinamide. After that, the N-dealkylated amine undergoes oxidation to a carboxylic acid and glucuronidation. CYPs (cytochrome P450s) mediate dealkylation processes, particularly CYP3A4.

2. MATERIALS AND METHODS:

Materials and Reagents:

Safinamide standard drug purchased from Vidisha Analytical laboratory and safinamide tablets (Xafinact 100 mg tablet) purchased from local pharmacy. Water, Acetonitrile, Methanol, orthophosphoric acid all HPLC- grade reagents employed in the current study. HPLC analysis was performed using the pharmaceutical formulation safinamide (label claim include100 mg). Rankem's HPLC-grade water was used in HPLC study.

Instrumentation and software:

An Agilent 1260 Infinity II HPLC system equipped with an autosampler and a UV detector was used as the chromatographic system. Chromatograms were recorded using the Open Lab EZ Chrome Workstation on a Windows-based computer for data collection and processing. Safinamide concentrations were measured using a Kromasil C18 column (250 mm × 4.6 mm ID, 5 µm).

Experimental work:

Chromatography:

A stationary phase with Kromasil 100 C18, 125 mm X 4.0 mm, 5 µm was selected with 0.2 % OPA and Methanol (65:35 v/v). The mobile phase flow rate was 1mL / min and the injection volume was 20 µL. UV detection was performed at 226 nm. These conditions gave us the sharp and symmetrical peak with retention time of 3.7 min.

3. 1. PREPARATION OF SOLUTION

A) Preparation of Mobile phase

Preparation of 0.2 % Orthophosphoric acid: Measure and transfer accurately about 2 ml of orthophosphoric acid in 1000 ml of water Mixed well. filter through 0.45µ nylon membrane disc filter and degas.

Preparation of Mobile phase: Prepare a mixture 0.2 % OPA and Methanol in the ratio of 65:35 v/v Mixed well.

Preparation of Diluent: Water: ethanol 40:60 % v/v

Preparation of Blank: Use diluent as blank.

B) Preparation of standard stock solution for Chromatographic development:

Weigh accurately about 34 mg of Safinamide mesylate (Equivalent 25 mg of Safinamide) working standard and transfer it into 50 mL amber coloured volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent.

Further dilute 5 mL each of Safinamide stock solution to 25 mL with diluent. (100 ppm Safinamide).

Preparation of Standard solution:

Preparation of Safinamide Standard stock solution: Weigh accurately about 34 mg of Safinamide mesylate (Equivalent 25 mg of Safinamide) working standard and transfer it into 50 mL amber coloured volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent.

Further dilute 4 mL each of Safinamide stock solution to 50 mL with diluent. (40 ppm Safinamide).

Preparation of Sample solution:

Take the 20 tablet and measure the average weight of tablet. Weighed 20 tablets and transferred in mortar pestle and crushed to fine powder. Mixed the contents uniformly. Weighed and transferred Xafinact 100 (Safinamide 100 mg) tablets powder Equivalent 200 mg of Safinamide in to 200 mL clean and dry volumetric flask. Added about 150 mL of diluent, sonicate for 60 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

Further dilute 2 mL of sample stock solution to 50 mL volumetric flask make up with diluent up to mark mixed well. (Concentration of Sample Solution: 40 ppm)

Final Conclusion: Water: Ethanol (20:80% v/v) will be used as a diluent for preparing stock solution.

4. RESULTS AND DISCUSSION:

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND OPTIMIZATION:

The following chromatographic conditions were established by trial and error.

Table 1: Chromatographic Conditions

Mobile phase	:	0.2 % OPA and Methanol (65:35 v/v)
Column	:	Kromasil 100 C18, 125 mm X 4.0 mm, 5 µm
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µL
Wavelength	:	226 nm
Column oven Temp	:	40ºC
Run time	:	7 minutes
Retention time	:	About 3.7 minutes for Safinamide

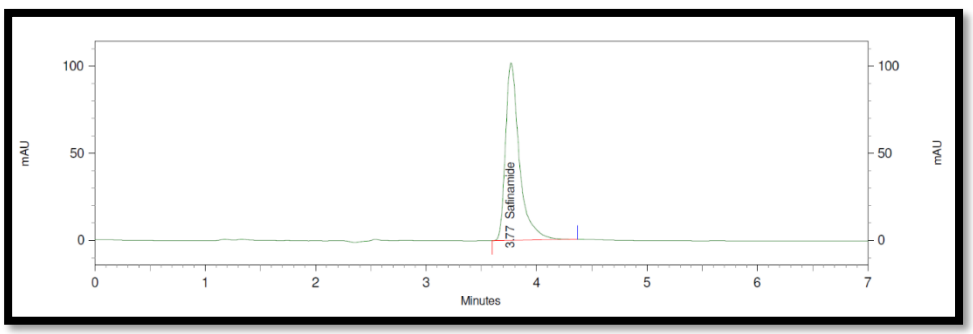

Fig. 2: Typical chromatogram for Safinamide

Observation: Safinamide eluted and good chromatography observed.

Conclusion: Above trial is selected as optimized chromatography because peak shape found good.

5. ANALYTICAL METHOD VALIDATION OF RP-HPLC

The established method for estimating safinamide for the following parameters was validated using ICH Q2(R1) recommendations. Specificity, linearity, accuracy, precision, and robustness were among these criteria. [6]

1. SYSTEM SUITABILITY: System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

Table 2: system suitability test of Safinamide

Tailing Factor	1.40
Theoretical plates	5842
Injection No.	Area
1	14052634
2	14100685
3	14119864
4	14069520
5	14055268
Mean	14079594
%RSD	0.21

The tests were performed by collecting data from Single injection of blank (Diluent) and five replicate injections of Standard solution were injected into the chromatograph. The data obtained is summarized in Table.

Conclusion:

The data demonstrates that the system suitability is within the acceptance criteria, thus

system is suitable.

2 Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table.

Table 3: Specificity (Identification and Interference)

<table>
<tr><td rowspan="2">Solution</td><td colspan="3">Specificity data</td></tr>
<tr><td>Retention time (min)</td><td colspan="2">Purity Match</td></tr>
<tr><td>Blank solution</td><td>NA</td><td colspan="2">NA</td></tr>
<tr><td>Placebo solution</td><td>NA</td><td colspan="2">NA</td></tr>
<tr><td rowspan="2">Standard solution</td><td rowspan="2">3.77</td><td>Purity angle</td><td>Purity threshold</td></tr>
<tr><td>5.84</td><td>7.36</td></tr>
<tr><td>Sample solution</td><td>3.76</td><td>5.22</td><td>6.72</td></tr>
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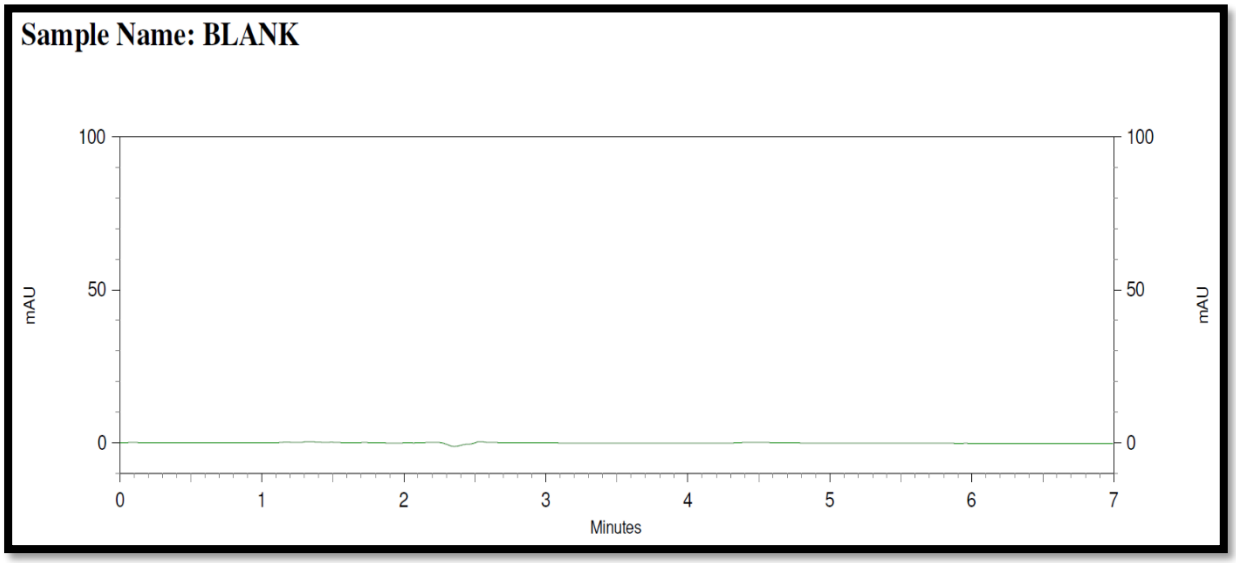

Fig. 3: Chromatogram of Blank

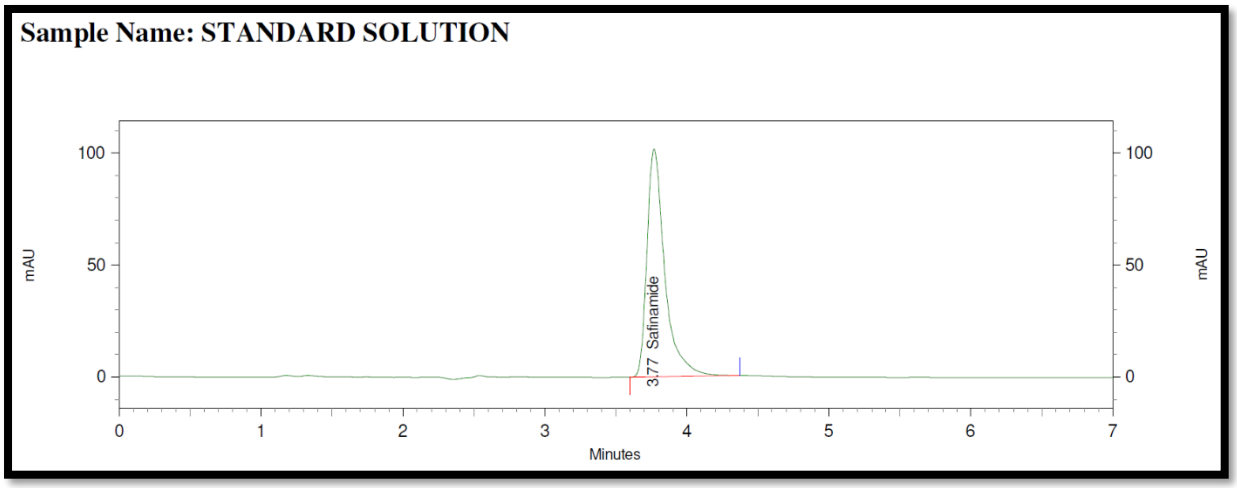

Fig. 4: Chromatogram of Standard

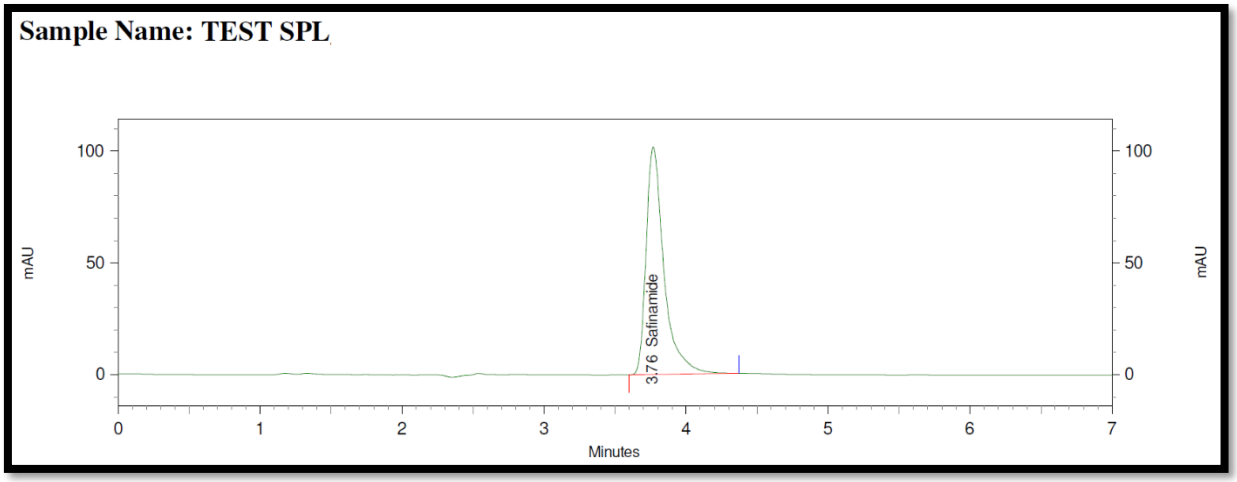

Fig. 5: Chromatogram of Sample

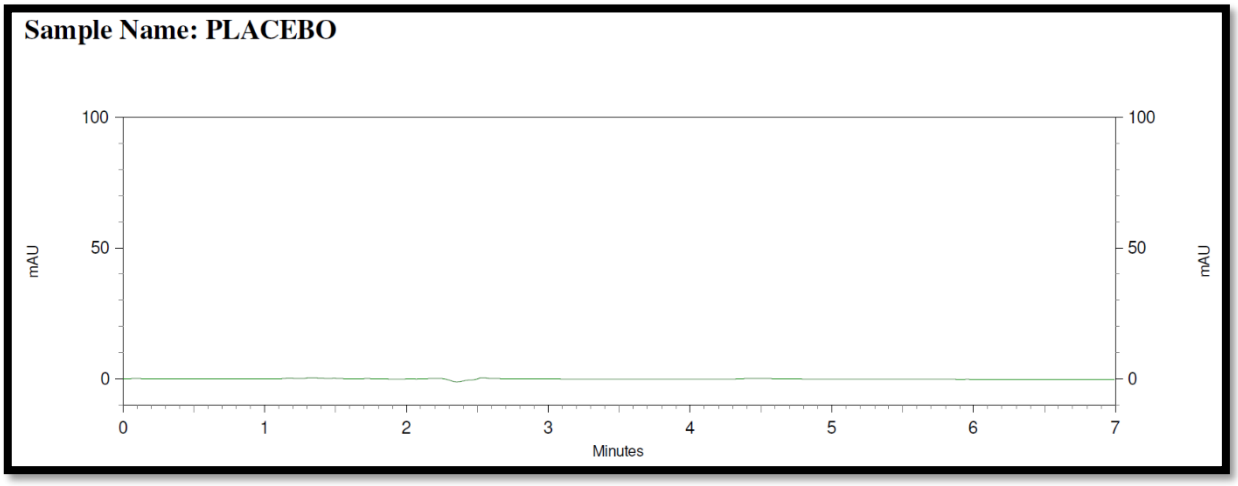

Fig. 6: Chromatogram of Placebo

CONCLUSION:

The data demonstrates that retention time in standard and sample is same for Safinamide peak.

The data demonstrates that there is no interference in blank and placebo at the retention time of Safinamide peak. Peak Purity match in both chromatograms obtained from Standard and Sample solution.

3. LINEARITY:

Linearity was evaluated in the range of 50 % to 150 % of Safinamide for working concentration. The working concentration of Safinamide in solution is 40 µg/mL. The data summarized in Table.

Table 4: Linearity of Safinamide

Level	Conc (µg/mL)	Area	Mean
50%	20	7059861	7059449
		7062524	
		7055961	
75%	30	10574183	10568262
		10568189	
		10562415	
100%	40	14025969	14040404
		14029418	
		14065824	
125%	50	17523051	17525489
		17520448	
		17532967	
150%	60	20958694	20952654
		20965418	
		20933851	

Corr. Coeff	1.000
Intercept	131797
Slope	347436
% Y-intercept	0.94

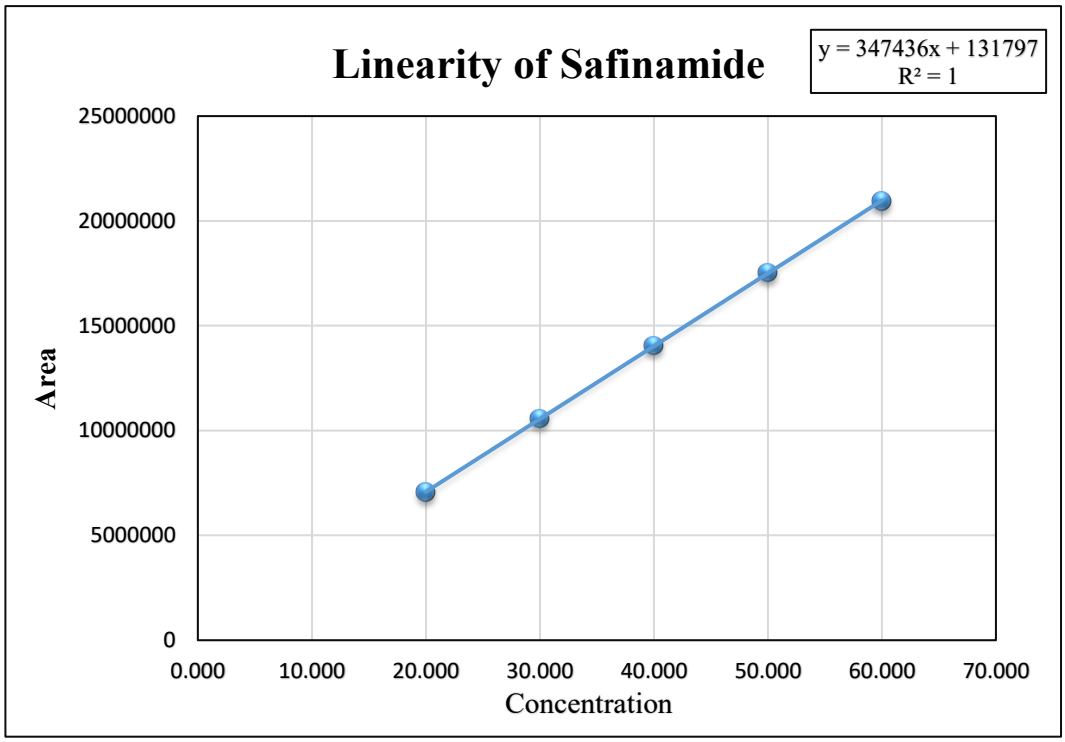

Fig.7: Linearity plot of Safinamide

CONCLUSION:

The data shows that system suitability is fulfilled.

The data shows that the response is found to be linear.

Co-relation coefficient (r^2 was found 1.0000)

4 Accuracy (Recovery):

Evaluated accuracy from 50% to 150% of Safinamide tablet, working concentration level. Each level prepared in triplicates.

Table 5: % Recovery for Safinamide

Level (%)	Area	Safinamide Added Conc (µg/mL)	Safinamide recovered Conc (µg/mL)	% Recovery	Mean % Recovery
50	7069851	20.49	20.51	100.89	100.37
	6925631	20.47	20.46	99.43	
	7106524	20.50	20.52	100.80	
100	14259634	40.00	40.07	101.28	99.92
	14099531	40.02	40.01	99.69	
	13950149	40.02	39.96	98.78	
150	21064851	60.51	60.48	99.69	100.06

	21126408	60.52	60.51	99.78	
	21235694	60.49	60.54	100.70	

CONCLUSION:

The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 98.0% - 102.0%.

5 Precision:

5.1 Method Precision:

Single injection of blank (Diluent), Standard solution (Five replicates) and sample solution (six preparations) was injected on the system.

Table 6: Method precision

Sample	Area	% Assay
Sample 1	13856934	98.45
Sample 2	13725905	97.29
Sample 3	13602968	96.27
Sample 4	13951261	99.44
Sample 5	13841529	98.34
Sample 6	13765908	97.73
Mean		97.92
STD DEV		1.0910
% RSD		1.114

CONCLUSION:

The data shows that system suitability is fulfilled.

The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

5.2 Intermediate Precision:

six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 7: Intermediate Precision

Sample	Area	% Assay
Sample 1	13658034	96.81
Sample 2	13852160	98.34
Sample 3	13705241	96.53
Sample 4	13785692	97.02

Sample 5	13852106	98.34
Sample 6	13912587	98.69
Mean		97.62
Std dev		0.9381
% RSD		0.961

Table 8: Intermediate Precision Pool Data

Parameter	Method Precision (Analyst-I)	Intermediate Precision (Analyst-II)
HPLC NO.	AD/HPLC-02	AD/HPLC-04
Column No.	HPLC-32	HPLC-37
Sample No.	%Assay	
1	98.45	96.81
2	97.29	98.34
3	96.27	96.53
4	99.44	97.02
5	98.34	98.34
6	97.73	98.69
Mean	98.45	97.62
Mean of Precision % Assay	97.77	
Absolute Mean difference % assay	1.0	

6. CONCLUSION:

The data shows that system suitability is fulfilled.

The data shows that % Assay is of six samples is not more than 2.0

The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

6.0 Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table 9: Robustness for Safinamide

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	13856934	NA
	1.1 ml/min	13816527	-0.3

Change in flow rate1.0 ml/min (±0.1 ml/min)	0.9 ml/min	13885008	0.2
Change in wavelength (±2 nm)	228 nm	13922109	0.5
	224 nm	13826854	-0.2

Conclusion:

System suitability criteria were fulfilled.

The difference of % assay value in each modified condition is within acceptance criteria

7. CONCLUSIONS:

The suggested approach demonstrated acceptable robustness, linear concentration range, accuracy, and precision. Based on the analysis results, the approach can be used to routinely determine the amount of Safinamide in pharmaceutical dosage forms and bulk drugs without interference from its excipients. This is also suited for determining Safinamide in tablet and bulk dosage forms.

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