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Analytical Method Development and Validation for Safinamide Mesylate using RP-HPLC with Quality by Design Approach

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To develop and validate a robust, stability-indicating RP-HPLC method for safinamide mesylate quantification employing systematic Quality by Design approach for pharmaceutical quality control and regulatory applications in Parkinson's disease therapy.Box-Behnken design with 17 experimental runs optimized three critical parameters: mobile phase composition (45-55% methanol), flow rate (0.8-1.2 mL/min), and column temperature (33-37°C). Chromatographic separation was achieved on Inertsil ODS-3V C18 column with UV detection at 226 nm. Response surface methodology established design space boundaries, followed by comprehensive validation per ICH Q2(R1) guidelines including specificity, linearity, accuracy, precision, robustness, LOD/LOQ, solution stability, and filter compatibility.Optimal conditions (47.5% methanol, 0.8 mL/min, 35°C) yielded retention time 3.56 minutes with excellent system suitability (% RSD 0.04%, theoretical plates 4366, asymmetry 1.11). Quadratic models demonstrated strong predictive capability (R^2 >0.88, relative errors <2%). The method exhibited exceptional linearity (r^2 = 0.9999, 2-30 μg/mL), high sensitivity (LOD 0.079 μg/mL, LOQ 0.240 μg/mL), excellent accuracy (99.81% recovery), superior precision (repeatability 0.86% RSD, intermediate 1.05% RSD), and robust performance under deliberate parameter variations. Solutions remained stable for 24 hours with both PVDF and Nylon filters compatible.

Keywords: Box-Behnken design; ICH Q2(R1); Method validation; Parkinson's disease; Quality by Design; RP-HPLC; Safinamide mesylate.

Parkinson's disease affects about 10 million people across the globe with its prevalence getting very high as one gets older with an average of 1-2 % of people aged above 60 years being affected by it.[1] The economic cost to the world is above 52billion each year which includes direct medical care costs and indirect costs through the cost of low productivity and support of caregivers.[2] Several challenges associated with current therapeutic approaches includeintricate polypharmacy schedules, inter-patient variability in the response to drugs and unreliable bioavailability characteristics.[3] Conventional analytical processes to track Parkinson drugs are oftentimes weak and do not consider important quality aspects in a systematic manner.[4] Increasing pressure is put on the pharmaceutical industry to come up with dependable, repeatable methods of analytical

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procedures to maintain the quality of the drugs throughout the product lifecycle.[5] The traditional method development methods are more of a trial-and-error methodology and they are time and resource consuming and offer minimal insight into the parameter of methods of great importance. Recent regulatory standards note the requirement of science based, risk assessed analytical processes thatprove the overall knowledge of the performance characteristics of methods and their scope of operation.[6]

Safinamide mesylate is a new type of monoamine oxidase-B (MAO-B) inhibitor that has two pharmacologic activities which are a combination of MAO-B inhibition with voltage-gated sodium channel blockage and calcium channel regulating activities.[7] This chemical compound has a molecular weight of 398.5 g/mol and the typical absorption maximum at 226 nm, which makes the compound visible in a liquid chromatography set. Clinical trials show that safinamide offers great benefits in motor fluctuations when combined with levodopa in adjunctive therapy to lengthening of on-time without bothersome dyskinesia by about 1-2 hours daily. The lipophilic property of the compound also allows easy access to the central nervous system tissues via blood-brain barrier with therapeutic concentrations.[8] Pharmacokinetics indicate the total absorption through the oral route, linearity in dose-proportional exposure and predominant hepatic metabolism by amide hydrolysis and glucuronidation. Published data determine the positive safety profile of safinamide with the least amount of dopaminergic side effects in comparison to conventional MAO-B inhibitors.[9]

MATERIALS AND METHODS

Materials

Safinamide mesylate reference standard (purity 99.8%) was procured from Sciquaint Innovations Pvt. Ltd., Pune, India. Methanol (HPLC grade, e"99.9%) and orthophosphoric acid (85% w/v, analytical grade) were obtained from Research Lab Fine Chem Industries, Pune, India. HPLC-grade water was prepared using a Milli-Q water purification system.

Methods

Determination of Absorption Maxima ($ë_{max}$) of Safinamide

A double-beam UV-visible spectrophotometer (Labindia Analytical Instruments Pvt. Ltd. Mumbai, India Model UV-3092) was used to ascertain the absorption maxima of safinamide. A stock solution was made by dissolving 13.18mg safinamide mesylate (synonymous to 10 mg safinamide base) in 20mL of water with the use of a sonic machine at 25 ± 2°C. The concentration was further diluted to 20 µg/mL by adding 0.8 mL of aliquot to 20 mL of water. The solution was scanned between 200-400nm against water as blank using 10mm quartz cuvettes at a scan rate of 200 nm/min. All the measurements were conducted three times (n=3) at room temperature.[10]

Chromatographic Conditions

The chromatographic process was conducted using a high-performance liquid chromatography (Agilent Technologies, Model 1260 Infinity II, USA) that had quaternary pump, autosampler, column thermostat and UV-visible detector. As a stationary phase, an Inertsil ODS-3V C18 column (150 mm × 4.6 mm i.d., 5 um particle size, GL Sciences Inc., Japan) was employed. The mobile phase was composed of 0.1 percent orthophosphoric acid in water (50:50, v/v) and it was isocratically added at a flow rate of 1.0 mL/min. During the analysis, the column oven temperature was held at 35°C. A 226 nm UV detector was used to perform detection. The volume of injection was fixed at 20 ìL and a run time of 10 minutes. Before analysis, 0.45 mu membrane filters and 15 minutes of sonication to remove any bubbles was done on the mobile phase. At least 30 minutes of incubation of the column in the mobile phase was allowed to stabilize the column before the initial injection in order to obtain the baseline stability and reproducible retention times.[11-13]

Quality by Design Approach

A Box-Behnken design as shown in Table 1 and Table 2 was employed for systematic optimization of the chromatographic method using Design-Expert® software (Version 7.0.0, Stat-Ease Inc., Minneapolis, MN, USA). The design consisted of 17 experimental runs with three independent variables: mobile phase composition

(X: 45-55% methanol), flow rate (X: 0.8-1.2 mL/min), and column oven temperature (X: 33-37°C), each evaluated at two levels (low and high). The dependent variables were retention time, peak asymmetry factor, and theoretical plates. Each run was performed in triplicate (n=3). The experimental data were analyzed using analysis of variance (ANOVA) and response surface methodology to establish relationships between variables, identify significant factors, and determine the optimal design space where method performance consistently met predefined acceptance criteria.[14-16]

RESULTS

Scanning absorbance maxima determination

UV spectrophotometric analysis showed that the highest absorption was at 226 nm as shown in Figure 1 and the wavelength was chosen as the detection wavelength in the method of HPLC. This wavelength gave sufficient sensitivity to detect safinamide and reduced any possible interference by the mobile phase components and excipients of pharmaceuticals. The large molar absorptivity of the solution at 226 nm helped in the high level of sensitivity of the method as the detection and quantitative limits were low. The wavelength used was in line with the chromophoric structure of safinamide that has an aromatic benzamide moiety that provides UV absorption in this wavelength range.

System Suitability Test

The system suitability test showed a very good chromatographic result with the area of the peak percentage of RSD of only 0.04% which is very low relative to the acceptance criterion of 2.0% give in Table 3. The theoretical plate count was 4366; demonstrating excellent column efficiency. The eventual asymmetry of the peak (1.11) provided evidence of the superb peak symmetry whereas the stability of the retention time (3.56 minutes) of six repeated injections indicated the stability of the chromatographic environment.

The Box-Behnken design with 17 experimental conditions was able to efficiently investigate the design space giving a range of retention times of between 1.72 and 5.27 minutes, a range of asymmetry factors of between 1 09 and 1.22 and a range of theoretical plates of 3083 to 4903 as given in Table 4. Excellent reproducibility and little variation were observed with center point replicates (run 9, 10, 13, 14, 17), thereby proving the reliability of the experiments. The acceptable values of peak asymmetry were found to be below 2.0 in all the runs and the theoretical plate was more than 2000 and passed the ICH standards.

Optimization

Effect on Retention Time

The quadratic model for retention time was highly significant (F-value = 22.77, p = 0.0002) with excellent fit (R^2 = 0.9245, adjusted R^2 = 0.8331, predicted R^2 = 0.7388). ANOVA revealed that methanol percentage (Factor A) was the most significant factor (F = 145.10, p < 0.0001), followed by flow rate (Factor B, F = 36.57, p = 0.0005). Column oven temperature showed no significant effect (p = 0.2535). The quadratic term A^2 was significant (F = 14.58, p = 0.0066), indicating curvature effect as given in Table 5 and Table 6.

Retention Time = 2.79 - 1.06A - 0.53B + 0.11C - 0.18AB + 0.065AC - 0.0075BC + 0.35A^2 + 0.12B^2 - 0.17C^2

Response surface plots demonstrated that retention time decreased significantly with increasing methanol percentage (45-55%), with the effect more pronounced at lower flow rates. The negative coefficients confirmed inverse relationships. Optimal retention time (2.5-3.5 minutes) was achieved at 47-50% methanol with flow rates of 0.9-1.1 mL/min.

Effect on Peak Asymmetry

The quadratic model for asymmetry was significant (F = 8.95, p = 0.0043) but showed moderate fit (R^2 = 0.5135, adjusted R^2 = 0.4135). Methanol percentage was the most significant factor (F = 37.56, p = 0.0005), followed by flow rate (F = 16.06, p = 0.0051) and temperature (F = 9.39, p = 0.0182). The quadratic term B^2 was significant (F = 8.45, p = 0.0228).

Peak Asymmetry = 1.14 - 0.028A + 0.018B + 0.014C - 0.010AB - 0.0000AC - 0.0125BC + 0.0105A^2 + 0.0140B^2 - 0.0032C^2

Contour plots Figure 2 showed that asymmetry remained stable (1.09-1.22) across the experimental domain, well within acceptable limits (d" 2.0). Optimal symmetry was achieved at higher methanol percentages (50-55%) with moderate flow rates (0.8-1.0 mL/min) and lower temperatures (33-35°C).

Effect on Theoretical Plates

The quadratic model was highly significant ($F = 14.55$, $p = 0.0010$) with good fit ($R^2 = 0.8840$, adjusted $R^2 = 0.8117$, predicted $R^2 = 0.7005$). Flow rate was the most significant factor ($F = 65.79$, $p < 0.0001$), followed by methanol percentage ($F = 50.57$, $p = 0.0002$). Temperature showed no significant effect ($p = 0.5150$). The quadratic term A^2 was significant ($F = 9.76$, $p = 0.0168$).

Theoretical Plates = 3923 - 402.50A - 459.25B + 12.25C + 9.25AB + 19.00AC + 47.00BC + 182.50A^2 + 76.63B^2 + 90.13C^2

Response surface plots revealed that maximum theoretical plates (>4500) were achieved at lower methanol percentages (45-47%) with lower flow rates (0.8-0.9 mL/min). Both negative coefficients indicated that increasing methanol and flow rate decreased column efficiency.

Validation of statistical models

The constructed quadratic models showed great predictability, and relative errors in the predicted and experimental values were 0.72% to 1.89% in all the three responses given in Table 7. Close correlation of predicted (3.494 min) and

Table 1. Experimental variables and their levels in Box-Behnken design for method optimization

Sr. No	Independent Variables	Levels	
		Low (-1)	High (+1)
1	Methanol (%)	45	55
2	Flow rate (mL/min)	0.8	1.2
3	Column oven temperature (°C)	33	37
	Dependent Variable		
	Retention time	Range (2-6)	
	Peak asymmetric factor	Minimize	
	Theoretical plates	Maximize	

Table 2. Experimental runs and design matrix for Box-Behnken optimization

Run	Factor A: Methanol (%)	Factor B: Flow rate (mL/min)	Factor C: Column oven temperature (°C)
1	50	1.2	32
2	55	1	32
3	55	0.8	35
4	50	0.8	32
5	45	0.8	35
6	45	1.2	35
7	50	1	35
8	55	1.2	35
9	50	0.8	38
10	50	1	35
11	45	1	32
12	45	1	38
13	50	1	35
14	50	1	35
15	50	1.2	38
16	55	1	38
17	50	1	35

experimental (3.56 min) retention times and slight differences in asymmetry factor and theoretical plates proved the suitability of Box-Behnken design to optimise chromatography. All the relative errors were not more than 5% which means that the mathematical models are credible in predicting the performance of methods in the design space and can be used to guide future method modification without further experimental work.

Results of Method Validation

Specificity Study

The technique was highly specific and this had no other interfering peaks at the retention time of safinamide at blank or placebo solutions as per Table 8. Both standard (0.982) and test solutions (0.975) had peak purities of over 0.95, indicating that the spectrometry was homogeneous and there were no impurities co-eluting. Chromatograms revealed clear safinamide peaks without excipients of the tablet such as diluents, binders and disintegrants.

Linearity study

The procedure showed a great linearity within the concentration of 2-30 ìg/mL (10-150% of the working range) with a correlation coefficient (R^2) of 0.9999, which is much better than the ICH acceptance limit of 0.999 depicted in Figure 3. The values of the % RSD were less than 0.3 % at each of the concentration levels which portrayed extraordinary precision of measurements. Linear regression equation y = 996180.6x - 5027.3 was good to be quantified with small intercept error.

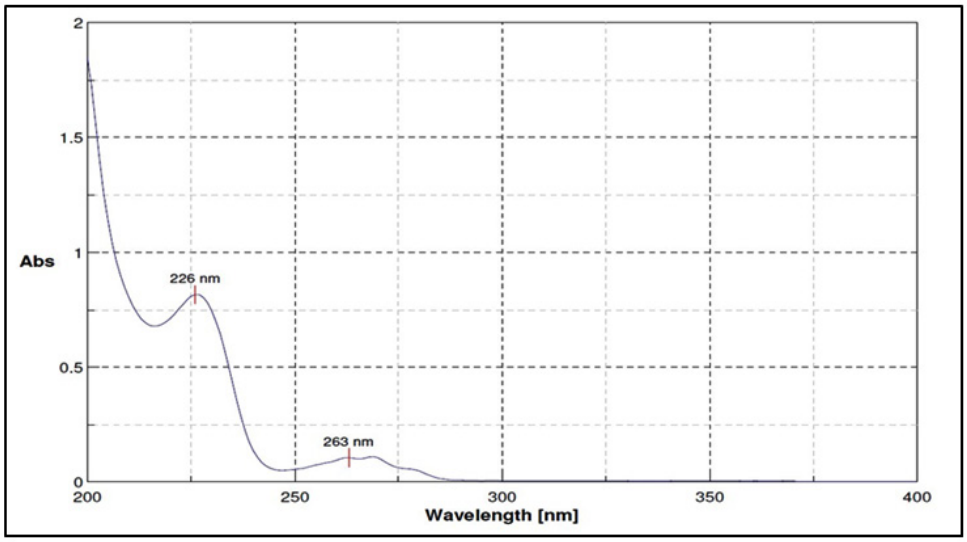

Fig. 1. Scanning absorbance maxima of safinamide (226 nm)

Table 3. System suitability test results for Safinamide

Injection No.	Peak Area	Retention Time (min)	Asymmetry	Theoretical Plates
1	19,946,358	3.56	1.11	4384
2	19,953,946	3.56	1.11	4351
3	19,942,861	3.56	1.11	4369
4	19,936,281	3.56	1.12	4351
5	19,952,864	3.56	1.11	4376
6	19,946,462	3.56	1.11	4366
Mean ± SD	19,946,462 ± 7308	3.56 ± 0.00	1.11 ± 0.00	4366 ± 14
% RSD	0.04	0.00	0.00	0.32

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The calculated LOD (0.079 ìg/mL) and LOQ (0.240 ìg/mL) values demonstrated high sensitivity of the developed method as per Table 9. The LOQ, representing only 1.2% of working concentration, indicated capability for trace-level quantification in pharmaceutical samples. These low detection limits, determined from calibration curve statistics following ICH Q2(R1) guidelines, enable accurate determination of safinamide even in degraded samples or low-dose formulations. The experimental verification of LOQ with % RSD below 2.0% confirmed adequate precision at this concentration level, establishing method suitability for impurity profiling and stability studies.

Accuracy (Recovery Studies)

The recovery studies demonstrated excellent accuracy with mean recoveries of 99.60%, 100.00%, and 99.82% at 50%, 100%, and 150% levels respectively, all within the acceptance range of 98-102% shown in Table 10. Overall mean recovery of 99.81% with % RSD of 0.91% confirmed method's ability to accurately quantify safinamide across the analytical range without matrix interference.

Precision

The method demonstrated excellent precision with % RSD of 0.86% for repeatability and 1.05% for intermediate precision, both well below the acceptance limit of 2.0% given in Table 11. Combined precision of twelve determinations yielded % RSD of 0.97%, confirming exceptional reproducibility across different days and analysts. All individual assay values ranged from 95.78% to 98.62%, within acceptable limits of 90-110%.

Robustness

The method remained unaffected by deliberate variations in chromatographic parameters, with all system suitability criteria maintained within acceptable limits shown in Table 12. Theoretical plates ranged from 3937 to 4453 (all >2000) and asymmetry values remained between 1.09-1.14 (all <2.0) across tested conditions.

Solution Stability

Both standard and test solutions demonstrated adequate stability at room temperature for 24 hours, with % differences of 0.39% and 0.55% respectively, well within the acceptance criterion of d"2.0% as per Table 13. The minimal degradation observed over 24 hours

Table 4. Experimental results of Box-Behnken design showing chromatographic responses

Run	Retention time (RT)	Asymmetry	TP
1	2.39	1.09	3267
2	2.16	1.1	3455
3	2.63	1.12	4191
4	3.56	1.12	4352
5	4.9	1.18	4818
6	3.27	1.13	3673
7	2.78	1.14	3784
8	1.72	1.11	3083
9	3.34	1.18	4237
10	2.78	1.14	3775
11	5.27	1.19	4741
12	4.87	1.22	4903
13	2.78	1.14	3793
14	2.79	1.14	3766
15	2.14	1.1	3340
16	2.02	1.13	3693
17	2.79	1.14	3773

Table 5. Model fit summary of retention time, peak asymmetry and theoretical plates

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Retention time					
Quadratic	0.0186	< 0.0001	0.9245	0.4717	Suggested
Peak asymmetric factor					
Quadratic	0.0506		0.8172	-0.2796	Suggested
Theoretical plates					
Quadratic	0.0442	< 0.0001	0.8840	0.1894	Suggested

Table 6. ANOVA summary of retention time, peak asymmetry and theoretical plates

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Retention Time						
Model	16.89	9	1.88	22.77	0.0002	significant
A-Methanol	11.96	1	11.96	145.10	< 0.0001	
B-Flow rate	3.01	1	3.01	36.57	0.0005	
C-Column oven temperature	0.1275	1	0.1275	1.55	0.2535	
AB	0.1296	1	0.1296	1.57	0.2501	
AC	0.0169	1	0.0169	0.2051	0.6643	
BC	0.0002	1	0.0002	0.0027	0.9598	
A^2	1.20	1	1.20	14.58	0.0066	
B^2	0.1492	1	0.1492	1.81	0.2204	
C^2	0.2885	1	0.2885	3.50	0.1035	
Peak Asymmetry						
Model	0.0181	9	0.0020	8.95	0.0043	significant
A-Methanol	0.0084	1	0.0084	37.56	0.0005	
B-Flow rate	0.0036	1	0.0036	16.06	0.0051	
C-Column oven temperature	0.0021	1	0.0021	9.39	0.0182	
AB	0.0004	1	0.0004	1.78	0.2242	
AC	3.469E-18	1	3.469E-18	1.542E-14	1.0000	
BC	0.0006	1	0.0006	2.78	0.1395	
A^2	0.0011	1	0.0011	4.94	0.0616	
B^2	0.0019	1	0.0019	8.45	0.0228	
C^2	0.0001	1	0.0001	0.2632	0.6237	
Theoretical Plates						
Model	4.462E+06	9	4.958E+05	14.55	0.0010	significant
A-Methanol	1.723E+06	1	1.723E+06	50.57	0.0002	
B-Flow rate	2.242E+06	1	2.242E+06	65.79	< 0.0001	
C-Column oven temperature	16020.50	1	16020.50	0.4702	0.5150	
AB	342.25	1	342.25	0.0100	0.9230	
AC	1444.00	1	1444.00	0.0424	0.8428	
BC	8836.00	1	8836.00	0.2593	0.6262	
A^2	3.325E+05	1	3.325E+05	9.76	0.0168	
B^2	58602.53	1	58602.53	1.72	0.2311	
C^2	81088.42	1	81088.42	2.38	0.1668	

confirms solution integrity during typical analytical sequences.

Filter Compatibility

Both PVDF and Nylon filters demonstrated excellent compatibility with minimal differences of 0.05% and 0.18% respectively compared to unfiltered samples, well within the 2.0% acceptance limit given in Table 14. The negligible variations confirmed absence of drug adsorption onto filter membranes and no interference from filter materials.

DISCUSSION

The present study successfully developed and validated a robust RP-HPLC methodof safinamide mesylate by the Quality by Design principles. Box-Behnken design effectively optimized the chromatographic conditions with little experimental input as compared to conventional methods, and the response surface methodology identified the composition of mobile phase and flow rate as important variables in performance. The UV

analysis showed the best wavelength to be 226nm, which has good sensitivity as indicated by low LOD (0.079 ìg/mL) and LOQ(0.240 ìg/mL). The obtained system suitability showed outstanding performance with a maximum area percentage RSD of 0.04%, theoretical plates of 4366, and an asymmetry of 1.11 . Box-Behnken design gave the retention time of 1.72-5.27 minutes, asymmetry 1.09-1.22, and hypothetical plates of 3083-4903 in 17 runs . Experimental reliability was determined

Table 7. Validation of statistical model

Batch	Response	Predicted value	Experimental value	% Relative error
Run 4	Retention time	3.494	3.56	1.89
	Peak asymmetric factor	1.112	1.12	0.72
	Theoretical plates	4298	4352	1.26

Table 8. Specificity study results

Description	Observation
Blank	No interference at retention time of safinamide
Placebo	No interference at retention time of safinamide
Standard solution (20 µg/mL)	Peak purity: 0.982
Test solution (20 µg/mL)	Peak purity: 0.975

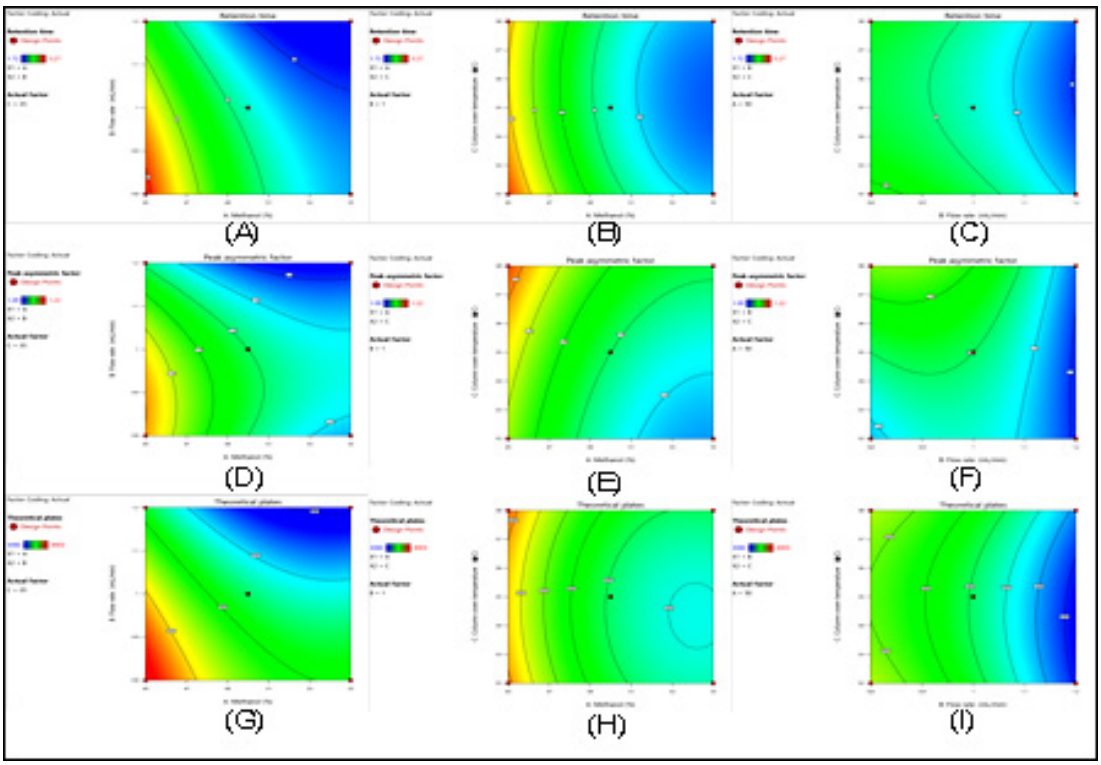

Fig. 2. Contour plots showing the effect of independent variables on chromatographic responses: (A-C) retention time, (D-F) peak asymmetry factor, and (G-I) theoretical plates as functions of methanol percentage, column oven temperature, and flow rate

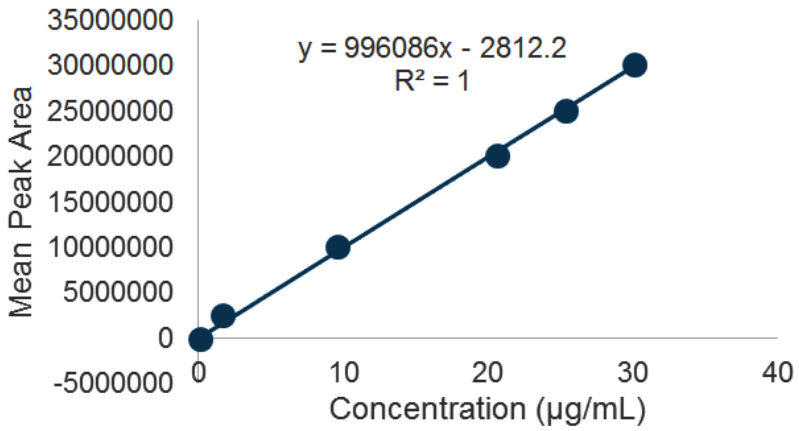

Fig. 3. Calibration curve showing linear relationship between concentration and peak area of safinamide

Table 9. LOD and LOQ determination for safinamide

Parameter	Formula	Value	Result
Residual standard deviation (σ)	From regression line	23,931.73	-
Slope (S)	From calibration curve	996,180.614	-
Limit of Detection (LOD)	3.3σ/S	(3.3 × 23,931.73) / 996,180.614	0.079 µg/mL
Limit of Quantitation (LOQ)	10σ/S	(10 × 23,931.73) / 996,180.614	0.240 µg/mL

Table 10. Accuracy (Recovery) Study of the Developed Method

Level (%)	Area	Recovered Conc. (µg/mL)	Added Conc. (µg/mL)	% Recovery	Mean % Recovery
50	9,956,341	9.98	10.01	99.70	99.60
	10,096,140	10.12	10.07	100.50	
	9,896,215	9.92	10.06	98.61	
100	19,953,622	20.01	20.01	100.00	100.00
	19,720,134	19.77	19.98	98.95	
	20,112,631	20.17	19.96	101.05	
150	29,551,091	29.63	30.01	98.73	99.82
	29,865,149	29.95	30.00	99.83	
	30,165,151	30.25	29.98	100.90	

with center point reproducibility and excellent fit was obtained with quadratic models (R^2 = 0.9245 retention time, 0.8840 theoretical plates).

The good predictive power of the model was shown by relative errors of 0.72-1.89% between predicted and experimental values, which shows that the model performs well in the design space. Extensive ICH Q2(R1) validation determined method suitability: specificity was found to be unaffected by excipients with peak purity of >0.95; linearity was found to have an R^2 = 0.9999 over a range of 2-30 ìg/mL); accuracy was found to yield a mean recovery of 99.81% with a RSD of 0.91%.The reliability of the methods was checked with robustness testing, which introduced known parameter changes in the method and kept the theoretical plates 3937-4453 and asymmetry 1.09-1.14 steady when the method is tested under different conditions There was a stability of the solution of less than 0.6% per 24 hours and filter

compatibility analysis indicated little change of 0.05-0.18% with PVDF and Nylon filters.

QbD offered rich design space knowledge with reduced experiments compared to factorial designs which allows versatile operation over established ranges and allows transfer of the methodology. This method is the best choice because of its short retention time (3.56 minutes), simple isocratic mobile phase, high sensitivity, and strength, which would be suitable in routine quality control, stability investigations, and dissolution experiments in pharmaceutical laboratories.

Table 11. Precision study results for safinamide tablet assay

Sample	Sample	Repeatability (Intra-day)		Intermediate Precision (Inter-day)	
	(mg)	Peak Area	% Assay	Peak Area	% Assay
1	125.3	19,526,415	97.85	19,524,181	97.68
2	125.6	19,251,632	96.24	19,425,814	97.50
3	126.1	19,256,314	95.89	19,235,017	95.78
4	125.9	19,256,381	96.04	19,238,174	96.48
5	124.5	19,354,182	97.61	19,536,189	97.82
6	125.2	19,256,814	96.58	19,757,873	98.62
Mean ± SD	-	-	96.70 ± 0.83	-	97.31 ± 1.02
% RSD	-	-	0.86	-	1.05

Table 12. Robustness evaluation: system suitability parameters under varied chromatographic conditions

Parameter Variation	Retention Time (min)	Peak Area	Asymmetry	Theoretical Plates
Wavelength +3 nm (229 nm)	3.35	19,909,884	1.14	4167
Wavelength -3 nm (223 nm)	3.34	17,959,112	1.11	4195
Flow rate +10% (0.88 mL/min)	3.03	17,854,000	1.11	3944
Flow rate -10% (0.72 mL/min)	3.70	21,920,761	1.12	4453
Temperature +2°C (34°C)	3.38	19,922,616	1.12	4295
Temperature -2°C (30°C)	3.49	19,951,407	1.09	3937

Table 13. Solution stability study results

Time Point	Sample Solution	% Absolute Difference	Time Point	Standard Solution	% Absolute Difference
Initial	19,432,563	NA	Initial	19,943,691	NA
12 Hours	19,385,267	0.24	12 Hours	19,905,263	0.19
24 Hours	19,326,506	0.55	24 Hours	19,865,294	0.39

Table 14. Filter compatibility study results

Sample Type	Peak Area	% Absolute Difference from Unfiltered
Unfiltered	19,436,851	-
PVDF Filter (0.45 ìm)	19,426,581	0.05
Nylon Filter (0.45 ìm)	19,402,534	0.18

CONCLUSION

An effective, sensitive and reliable RP-HPLC methodology to quantify safinamide mesylate was effectively designed and validated using systematic Quality by Design. Box-Behnken design was able to optimize chromatography efficiently obtaining a well-defined design space with thorough knowledge of the important method parameters. The established procedure had superior linearity (R^2 = 0.9999), sensitivity (LOQ 0.240 ìg/mL), accuracy (99.81 % recovery), and precision (percent RSD <1.1 %), and could withstand purposeful variations in parameters, all in compliance with ICH Q2(R1) requirements. This method is economically feasible and environmentally friendly due to the short analysis time (3.56 minutes), the simplicity of its phase composition, and the low amount of solvent used to conduct the quality control of pharmaceuticals regularly. This is a validated technique that is easily applicable in drug companies to determine assays, stability testing, dissolution testing as well as regulatory filings of safinamide preparations to guarantee uniform product quality and patient safety in management of Parkinson disease.

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Conflicts of Interest

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Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable.

Author Contributions

Mayur Bhosale: Conceptualization, methodology, investigation, data curation, writing—original draft preparation; Rahul Khaire: Supervision, validation, formal analysis, writing—review and editing, project administration; Mohit Wagh: Methodology, experimental work, data analysis; Dheeraj Chechare: Resources, software, data interpretation; Yogesh Thombare: Visualization, validation, review of manuscript.

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