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Development and Validation of Stability Indicating RP-UPLC Method for Quantitative Estimation of Safinamide Mesylate in Bulk and its Tablet Dosage Form

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Abstract

Currently, there was an increasing interest on the development of a simple, rapid and sensitive method for safinamide mesylate due to its well- documented anti parkinsonism activity. This study aims to develop and validate a UPLC method for determination of the Safinamide mesylate in bulk and its tablet dosage forms. The chromatographic separation was achieved by using an ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 µm; Waters), with an isocratic elution of 0.02 M diammonium hydrogen phosphate buffer pH 9.0 and Acetonitrile (80:20 v/v), at a flow rate of 0.25 ml/min with the help of UV detection at 272nm. The results of the analysis were validated statistically as per the International Conference on Harmonization (ICH) guidelines. Linearity studies were carried out in the range of 10 - 60 µg/ml and the linear response (r^2) was found to be 0.9999 with limits of detection and quantification being 0.081 and 0.271µg, respectively. The precision was performed by analysis of standard and sample solutions of SAF at working concentration level for six times. The % RSD values of the system and method precisions were found to be 0.527 and 0.324 respectively. Then, the precision of the method was confirmed by intra-day and inter-day analysis. The % RSD value of the intra-day and inter-day precisions were found to be 0.324, 0.531 respectively. Recovery studies were performed for determining accuracy of the method and the percentage recovery was found to be 99.48-100.85%. The Robustness were performed at different flow rates and different temperatures, and the % RSD value were found to be 0.5965, 0.6276 respectively. Thus, a highly sensitive, simple and the stability indicating method were developed for the estimation of SAF in bulk and tablet dosage forms.

Keywords: Safinamide Mesylate, ICH Guidelines, ACQUITY BEH C18 Column, Ultra Performance Liquid Chromatography, Diammonium Hydrogen Phosphate Buffer

Introduction

Safinamide Mesylate (SAF) is a novel sodium and calcium channel blocker, capable with selective and reversible inhibition of monoaminooxidase type B (MAO-B) (1-7), chemically, it is (S)-(+)-2-[4-(3-fluoro benzyloxy benzyl amino) propanamide] methane sulfonate. (Figure: 1) which acts as Neuro protective with antiparkinsonian and anticonvulsion activity for the treatment of Parkinson's disease (8-11). Along with these activities, a well documented literature reports that there are few analytical methods like HPLC [12-14], HPTLC [15], LC-MS/MS (16, 17) are available for quantitative estimation and therapeutic effectiveness of SAF in bulk as well as formulation.

The development and validation of analytical methods for the accurate detection and quantification of active compounds in

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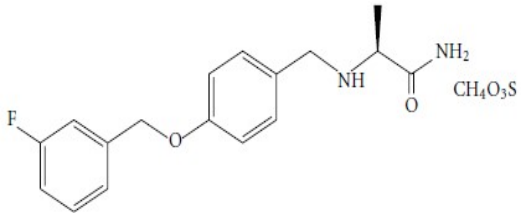

Figure: 1. Safinamide Mesylate

pharmaceutical samples with absence of interference of degradation products are a key consideration in the pharmaceutical field. Assay of SAF was mainly focused on its quantification by UPLC, mainly due to reward in terms of sensitivity and accuracy. The use of UPLC technology has been proposed to get out of the drawbacks like reducing the time of analysis and accordingly decreasing the environmental impact by reducing solvent consumption.

Herein we described the UPLC method development and validation of SAF in bulk and its tablet dosage forms for quantification and it was optimized and validated as per the ICH guidelines (18-20).

Material and Methods

Chemicals and Reagents: The SAF reference standard with a purity greater than 98% was gratis from Radiant Pharma, Mumbai, India. SAF tablets were purchased from commercial stores within their shelf life period. The reagents and solvents used (Acetonitrile, diammonium hydrogen phosphate) were of AR grade obtained from Merck Chemicals, Mumbai, India.

Instrumentation and UPLC Conditions: The estimation of SAF was performed using Waters' Acquity UPLC system (Waters, Milford, MA, USA) equipped with a quaternary gradient pump, auto sampler, column oven, and photodiode array detector and empower 2 software was used for analysis. An ultrasonic device, a sensitive balance, Sartorius analytic balance and a pH meter, glass electrode, were used for the preparation of solutions. Thermo Scientific Heraeus microbiological incubator, Digital Dry Baths, Labnet International and Spectroline E-Series UV lamp were used for stability studies.

0.02 M diammonium hydrogen phosphate and acetonitrile in a ratio of 80:20 v/v was selected as the mobile phase and the pH was adjusted by adding liquid ammonia (pH=9). The detector wavelength was set at 272nm. The flow rate is maintained at 0.25 ml/min, at an ambient column temperature with 5 µL injection volume.

Preparation of Mobile Phase: Freshly prepared solutions of 200 ml acetonitrile and 800 ml of 0.05 M ammonium acetate are transferred into a 1000ml standard flask and mixed well. Adjust the pH to 9 by adding a liquid ammonia solution with constant stirring and then filtered through 0.45 mm membrane filters.

Preparation of Stock Solution: The stock solution of SAF was prepared by taking 100mg of standard and transferred into a 100 ml standard flask having mobile phase and stirred continuously about 15 to 30 min. Finally made the final volume with the same solution to get the desired concentration (1mg/mL).

Preparation of Standard Solutions: 4 ml of stock solution of SAF was transferred with a calibrated pipette into a 100 ml flask. The final volume was made with the diluent to get 40µg/mL.

Preparation of Sample Solution: 20 tablets of SAF were weighed, powdered and transferred 673.45mg of powder which is equivalent to 100 mg of SAF into a 100ml standard flask with the mobile phase. Mix thoroughly using a stirrer for half an hour and made the final volume and filtered through a 0.45 mm filter. Further dilutions were made with the same diluent to get the optimum concentration of 40µg/mL.

UPLC Method Validation: UPLC developed method was validated by performing specificity/ selectivity, linearity, precision, accuracy, stability and robustness according to ICH guidelines for the estimation of SAF in bulk and tablet dosage form.

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Linearity: Different dilutions were prepared in the concentration range of 10-60 µg/ml of the stock solution (1mg/mL) of SAF. A standard curve was plotted by taking the peak area on 'y' axis and concentrations on 'x' axis. Regression analysis was used to evaluate the linearity of the method by the least square method.

Precision: The precision was performed by estimating standard, sample solutions of SAF (40µg/ml) at working concentration level for 6 times. Further the precision of the method was confirmed by the analysis of the formulation for three times in a day and one time in the three successive days.

Accuracy: Accuracy was established by recovery studies, carried out by spiking a known amount of SAF at three levels (20, 40 and 60µg/ml) to the tablet excipients. The sample solutions were analyzed in triplicate at each level and percentage recovery was calculated. The accuracy was evaluated based on the correlation between experimental value and theoretical value.

Robustness: The Robustness of the method was performed by altering the parameters like flow rate, column temperature. The method was analyzed at different flow rates and at different column temperatures using working standard and sample solutions of SAF.

Specificity: The specificity was verified by comparing chromatograms of the standard solution, matrices spiked wth SAF and a solution containing only matrices (blank samples). In order to confirm the presence or absence of interferences from matrices, the peak corresponding to SAF was analyzed and identified in each spiked matrix by UV spectra between 250 and 370nm, peak purity and retention time (from UPLC).

Forced Degradation: Forced degradation studies were also performed to know the stability of SAF. Acid hydrolysis and alkaline hydrolysis were investigated by adding hydrochloric acid (0.1 M HCl) and sodium hydroxide (0.1 M NaOH) to the standard solution of SAF, in order to prevent temperature and photolytic degradation, these solutions were kept at 25^{o}C and protected from light. After different periods of exposure, solutions were neutralized and analyzed. To evaluate peroxide degradation, to the standard solution hydrogen peroxide (3% H_2O_2) was added. UV degradation was performed by placing the standard solution in a UV chamber (250-370nm) with controlled temperature (25^{o}C). To assess the thermal degradation, standard solutions were exposed to 70^{o}C.

Results and Discussion

***Method Development*:** Detection and quantification of SAF have been analyzed by using different methodologies including chromatography and spectroscopy. Most of chromatographic methods are time consuming. In this study, UPLC method was selected based on its ability to endorse ultra pressure analysis and less time consuming with high precision, therefore increasing analysis efficiency.

Optimization of UPLC Method: Initially, different ratios of mobile phase, different column were tried for better separation of SAF. After performing various trials, finally mobile phase with the ratio of 80:20 %v/v of 0.02 M diammonium hydrogen phosphate buffer (pH 9.0) and acetonitrile and Waters Acquity BEH C18, 50 × 2.1 mm, 1.7µm column were selected for analysis as they produced a sharp and symmetrical peak with a retention value of 0.285 at 272nm. The flow rate was maintained all over analysis at 0.25 ml/min at ambient column temperature with the injection volume was 5 µL (Figure: 2).

Optimized Chromatographic Conditions: UPLC method development and validation of SAF was carryout by using Waters Acquity BEH C18, 50 × 2.1 mm, 1.7µm column, 0.02 M diammonium hydrogen phosphate buffer pH 9 and acetonitrile as mobile phase in the ratio of 80:20 v/v. Detection was done using a photo diode array detector at 272 nm. The flow rate was

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optimized to 0.25 ml/min at ambient temperature.

Application of Proposed Method to Tablet Formulation: To determine the concentration of SAF in tablets (Label claim: 100 mg per tablet), the contents of 20 tablets were weighed, finely powdered and their mean weight was determined. The powder equivalent to 100 mg of SAF was weighed and extracted with 100 ml of mobile phase; it was sonicated for 20 min. The resulting solution was filtered using 0.41µm filter.

Method Validation: The proposed method was validated as per the ICH guidelines in terms of its linearity, accuracy, specificity, Intraday and interday precision, robustness, ruggedness, LOD and limit LOQ.

Linearity (Calibration Curve): For linearity study, aliquots of 10, 20, 30, 40, 50, and 60 µg/ml of SAF from standard stock solution were injected into the waters acquity system (Figure: 3). The calibration curve was plotted using concentration against peak area and analyzed through least squares regression. The assay was found to be linear in the range from 10 -60µg/mL. The calibration curve was linear with an average correlation coefficient of r^2 0.999. Hence the selected

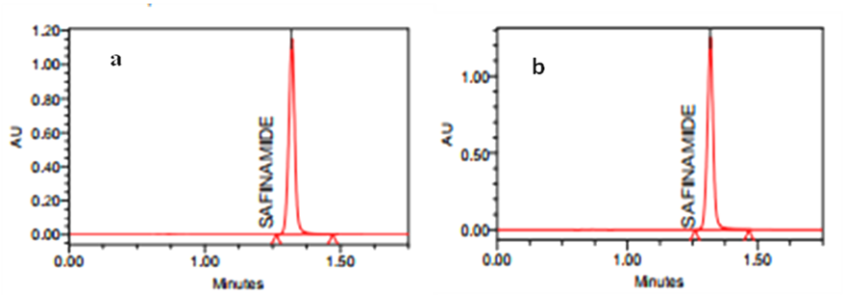

Figure: 2. Chromatograms of SAF Standard (a) and Sample (b)

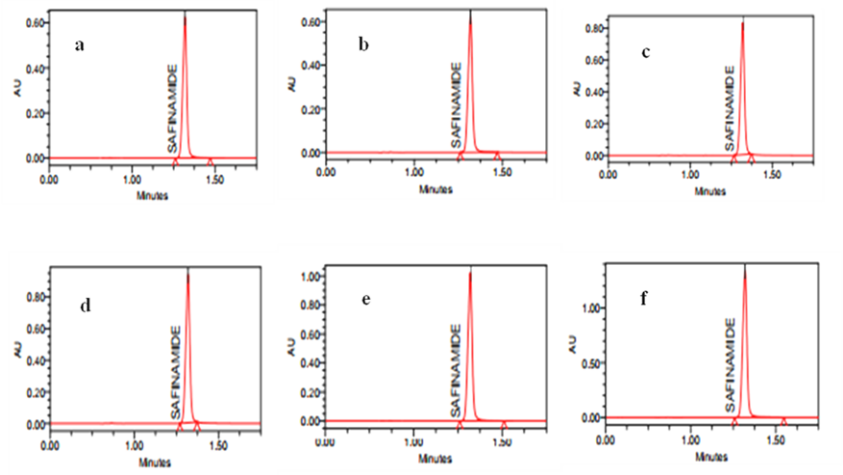

Figure: 3. Linearity Chromatograms of SAF; 10 µg/ml (a), 20 µg/ml (b), 30 µg/ml (c), 40 µg/ml (d), 50 µg/ml (e) and 60 µg/ml (f)

RP-UPLC Method

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concentrations were found to be linear (Figure: 4). Results are shown in Table: 1.

Accuracy: The accuracy of the method was performed by recovery studies. A known quantity of Safinamide mesylate raw material solutions was added at different levels (50,100 and 150%). The peak areas of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.85–100.11% (Figure: 5). Results of recovery data were shown in (Table 2).

Specificity: The specificity of the method was ascertained by analyzing blank, placebo, standard solution and tablet formulation. There were no interferences between excipients and Safinamide mesylate. Hence the method was specific (Figure: 6).

Precision: The precision was performed by analyzing standard and sample solutions of Safinamide mesylate at working concentration level for 6 times. The % RSD value of system precision and method precision were found to be 0.527 and 0.324 respectively. The amount present in tablet formulation was in good concord with the label claim. The results showed that the precision of the methods was confirmed.

Further the precision of the method was confirmed by intra-day and inter-day analysis. The analysis of the formulation was carried out for three times in the same day and one time in the three consecutive days. The results were shown in (Table: 3).

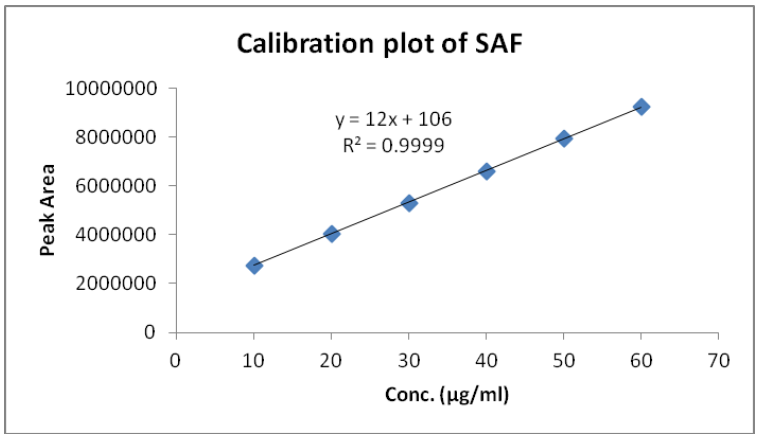

Figure: 4. Calibration Plot of SAF

Table 1: Linearity Data of SAF

S No	Linearity Level	Concentration (µg/ml)	Area
1	25	10	2756378
2	50	20	4042136
3	75	30	5296681
4	100	40	6594914
5	125	50	7923328
6	150	60	9236630
Correlation Coefficient			0.9999

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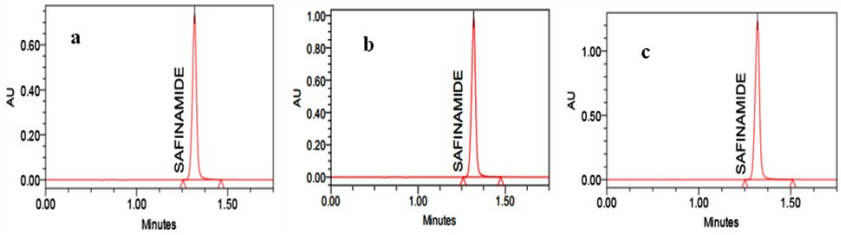
Robustness: The Robustness were performed at different flow rates (0.2ml/min, 0.25ml/min and 0.3ml/min.) and at different column temperatures (20˚C, 25˚C, and 30˚C) by using working standard and sample solutions of Safinamide mesylate. . The % RSD value of flow rate variation and column temperature variation were found to be 0.5965 and 0.6558 respectively (Figure: 7). Results are shown in Table: 4.

Limit of Detection (LOD) and Limit of Quantification (LOQ): In order to determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. SAF solutions of 10,



Figuure: 5. Accuracy Chromatograms of SAF; 50% (a), 100% (b) and 150% (c)

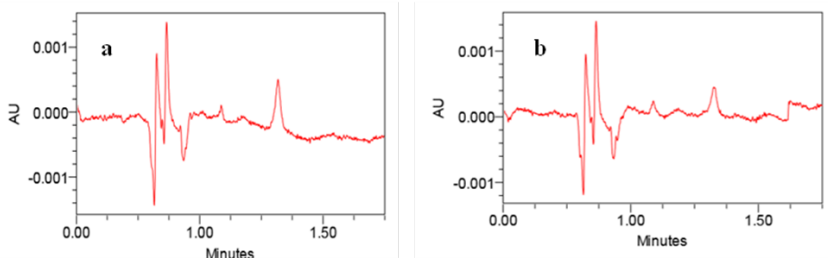


Fig 6. Chromatograms of Blank (a), Placebo (b)

Table: 2. Accuracy Data of SAF

Sample No	Spiked Level	Sample Weight (mg)	Sample Area	µg/ml Added	µg/ml Found	% Recovery	% Mean Recovery
1	50%	33.673	3631749	20.07	20.12	99.75	99.91
2		33.673	3611606	20.07	20.01	100.29	
3		33.673	3630428	20.07	20.13	99.70	
1	100%	67.345	6761150	40.13	40.15	99.95	100.11
2		67.345	6781061	40.15	40.14	100.02	
3		67.345	6805563	40.23	40.22	100.02	
1	150%	101.018	7304679	60.20	59.96	100.40	99.85
2		101.018	77375024	60.20	60.26	100.09	
3		101.018	7663028	60.20	59.64	99.06	

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Table 3. Method Precision, System Precision, Inter Day Precision Data of SAF

S No	Method Precision		System Precision		Inter Day Precision Data	
	Peak Area	% Assay	Peak Area	% Assay	Peak Area	% Assay
1	7222699	100.07	7219579	100.01	7278394	100.84
2	7242606	100.34	7289022	101.00	7283002	100.90
3	7218974	100.01	7289022	101.00	7293728	101.05
4	7276367	100.81	7216189	100.00	7209387	99.88
5	7265881	100.66	7216189	100.00	7218593	100.01
6	7232006	100.20	7214002	99.90	7218940	100.01
Average		100.35		100.31		100.45
SD		0.325		0.529		0.533
%RSD		0.324		0.527		0.531

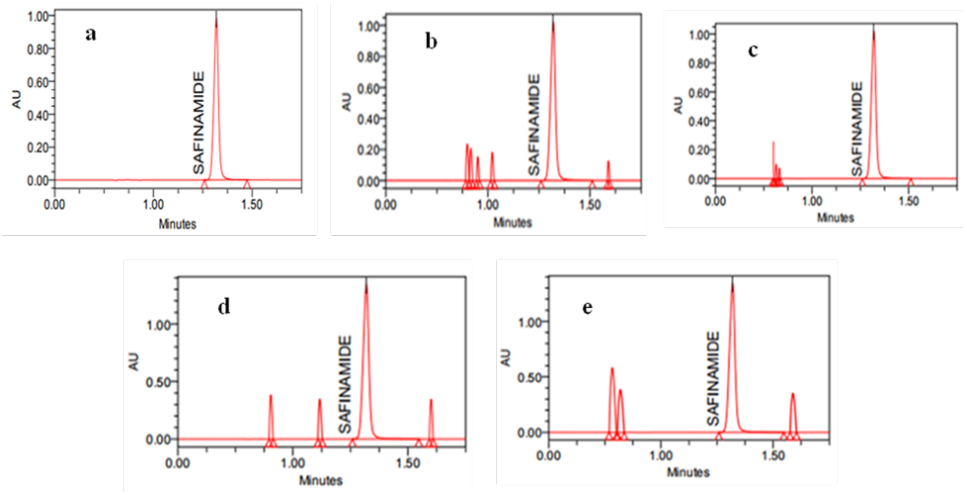

Figure: 7. Chromatograms of Untreated SAF (a), Acid-Degraded (b), Alkali-Degraded (c), Peroxide-Degraded (d), UV Light-Degraded (e)

20, 30, 40, 50, and 60µg/ µL were prepared and applied in triplicate. The LOQ and LOD were calculated using equation LOD = 3.3×N/B and LOQ = 10×N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve. Results are shown in Table: 5.

Degradation Studies

Stress Degradation Studies: The specificity of the method can be demonstrated through forced degradation studies performed under acidic, basic, oxidative, and ultraviolet light conditions. A specific method should be able to separate and equivocally identify the test compound from the various degradation products

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(Fig. 7). All experiments were conducted in triplicate (n=3). Results were shown in Table: 6.

Acid Hydrolysis: SAF (40 µg/ml) was prepared in HCl (1 M). Aliquots were kept at 25 °C for 24 h (Thermo Scientific Heraeus microbiological incubator, USA) and 80°C (AccuBlock™ Digital Dry Baths, Labnet international, USA) for 12 h.

Base Hydrolysis: SAF (40 µg/mL) was prepared in NaOH (1 M). Aliquots were kept at 25 °C for 24 h (Thermo Scientific Heraeus microbiological incubator, USA) and 80 °C (AccuBlock™ Digital Dry Baths, Labnet international, USA) for 12 h.

Oxidative Degradation: SAF (40µg/mL) was prepared using 30% (v/v) hydrogen peroxide (H_2O_2). Aliquots were incubated at 25 °C for 24 h and 80 °C for 12 h.

UV Degradation: SAF (40µg/mL) was prepared in the mobile phase and aliquots were kept in clear plastic vials to avoid unwanted UV absorption which may occur with glass vials. Samples were then exposed to UV light (365nm, Spectroline E-Series UV lamp, Spectronics Corp, USA) for a duration of 7 h.

Table 4. Robustness Data of SAF

S No	Parameter	Change	Peak Area	% Assay
1	Flow Rate (ml/min)	0.2	7157652	99.16
2		0.25	7243089	100.35
3		0.3	7208459	99.87
4	Temp (°C)	20	7242750	100.24
5		25	7250341	100.45
6		30	7325339	101.49

Table: 5. Analytical Performance Summary Data of SAF

S No	Validation Parameter	Results	Acceptance Criteria
1	Accuracy (%Recovery) (n=9)	Mean Recovery 101 %	% Recovery - 98% to 102%
2	Precision (n=6)	Mean assay -100.35 % % RSD – 0.324	Mean assay – 98% -102% % RSD should be < 2
3	Linearity	y = 12x + 106 R^2 = 0.9999	R^2 = 0.995
4	Degradation Studies (Acid, Base, Light, Peroxide)	Mean Assay – 90.775% % RSD – 1.619	The assay should be between 85-115% % RSD should be < 2
5	Limit of Detection	0.1454 µg/ml	- -
6	Limit of Quantification	0.4408 µg/ml	- -

Table: 6. Degradation Studies in Different Stress Conditions

Nature of the Sample	Sample Area	% Assay	Difference of Assay
Acid	4242136	89.25	10.75
Base	4273248	90.64	9.36
Peroxide	5696681	92.78	7.22
UV	5526886	90.43	9.57

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Discussion

SAF is a novel drug with multiple actions to treat Parkinson's disease which is a neurodegenerative disease. A stability indicating UPLC method was developed and validated for the quantitative determination of a Safinamide in tablet dosage form. The optimum concentration of SAF was found to be 40µg/ml. The chromatographic separation was achieved by using an ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 µm; Waters, USA), with an isocratic elution of 0.02 M diammonium hydrogen phosphate buffer pH 9.0 and Acetonitrile (80:20, v/v), at a flow rate of 0.25 ml/min with UV detection at 272nm. The results of the analysis were validated statistically as per the ICH guidelines. Linearity studies were carried out and the linear response (r^2 = 0.9999) was observed in the range of 10 - 60µg/mL with limit of detection (LOD) and quantification (LOQ) being 0.081 and 0.271 µg, respectively.

Precision was performed by injecting six replicate solutions of Safinamide standards and samples having the concentration of 40µg/ml. The % RSD value of system precision and method precision were found to be 0.527 and 0.324 respectively. The accuracy of the method was performed by recovery studies. The percentage recovery was found to be in the range of 99.48–100.85%. The Robustness were performed at different flow rates and different temperatures, and the % RSD value were found to be 0.5965, 0.6276 respectively. Hence the proposed method was successfully applied to a routine analysis of Safinamide mesylate in bulk and in tablet dosage form. Analytical performance summary data of Safinamide mesylate are shown in Table 6.

Conclusion

In the present study, a simple, rapid, sensitive and accurate UPLC method was successfully developed and validated for the quantitative determination of Safinamide in bulk and tablet dosage form. The results demonstrated a highly sensitive and selective method, without any interferences from the matrices and degradation products, able to quantify Safinamide mesylate with precision, accuracy and robustness. Hence it was concluded that the developed analytical method was successfully used for the routine quality control analysis of the Safinamide in marketing tablet dosage form.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Deeks ED., Safinamide: first global approval Drugs. 75 (2015) 705-11.

2. US Food and drug administration. “FDA approves a drug to treat Parkinson's disease” FDA news release 2017.

3. Marzo A., Dal Bo L., Monti N. C. (2004). Pharmacokinetics and pharmacodynamics of safinamide, a neuroprotectant with antiparkinsonian and anticonvulsant activity, Pharmacological Research. 50 (1): 77–85.

4. Caccia C, Maj R, Calabresi M (2006). Safinamide: from molecular targets to a new anti-Parkinson drug, Neurology. 67: S18-23.

5. Caccia C, Salvati P, Rossetti S, Anand R (2007). Safinamide: beyond MAO-B inhibition. Parkinsonism Relat Disord. 13: (Suppl 2) S99.

6. Onofrj M., Bonanni L., Thomas A. (2008). An expert opinion on safinamide in Parkinson’s disease, Expert Opinion on Investigational Drugs. 17(7): 1115–1125.

7. Schapira AH, Stocchi F, Borgohain R (2013). Long-term efficacy and safety of safinamide as add-on therapy in early Parkinson’s disease, Eur J Neurol. 20: 71-80.

8. Schapira AH, Fox S, Hauser RA 2013. Safinamide add on to Ldopa: a randomized, placebo-controlled, 24-week global trial in patients with Parkinson’s Disease (PD) and

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10.5530/ctbp.2022.3s.62

Motor fluctuations (SETTLE), 65th AAN Annual Meeting, American Academy of Neurology.

9. Gregoire L, Jourdain VA, Townsend M (2013). Safinamide reduces dyskinesias and prolongs L-DOPA antiparkinsonian effect in parkinsonian monkeys. Parkinsonism Relat Disord. 19 (5): 508-14.

10. Borgohain R, Szasz J, Stanzione P (2014). Randomized trial of safinamide add-on to levodopa in Parkinson's disease with motor fluctuations, Mov Disord, 29: 229-237.

11. Kandadai RM, Jabeen SA, Kanikannan MA, Borgohain R (2014). Safinamide for the treatment of Parkinson's disease, Expert Rev Clin Pharmacol, 7(6): 747-59.

12. Dal Bo L., Mazzucchelli P., Fibbioli M., and Marzo A. (2006). Bioassay of safinamide in biological fluids of humans and various animal species, Drug Research., 12: 814–819.

13. Zhang K., Xue N., Shi X., Liu W., Meng J., and Du Y. (2011). A validated chiral liquid chromatographic method for the enantiomeric separation of safinamide mesilate, a new anti-Parkinson drug, Journal of Pharmaceutical and Biomedical Analysis, 55(1): 220–224.

14. Mali Bhushan J., Vivekkumar K., Redasani K., Amod S. Patil, Atul Shirkhedkar A. (2013). Development and validation of RP-HPLC method for determination of safinamide mesylate in bulk and in tablet dosage form, Analytical Chemistry An Indian Journal, 13(4): 127-130.

15. Vivekkumar K., Redasani K., Mali Bhushan J., and Sanjay J. Surana (2012). Development and Validation of HPTLC Method for Estimation of Safinamide Mesylate in Bulk and in Tablet Dosage Form, International Scholarly Research Network Analytical Chemistry, 1-4.

16. Gotze L., Hegele A., Metzelder S.K. (2012). Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma, Clin. Chim. Acta., 413: 143–149.

17. Zou Liang, Sun Lili, Zhang Hui, Hui Wenkai, Zou Qiaogen, Zhu Zheying (2017). Identification, Characterization, and Quantification of Impurities of Safinamide Mesilate: Process-Related Impurities and Degradation Products. Journal of AOAC International, 100 (4): 1029-1037.

18. FDA, ICH-Q2 (R1): Validation of Analytical Procedures: Text and Methodology, vol. 60, US Food and Drug Administration, DC, USA, 1995.

19. FDA, ICH-Q1A (R2): Stability Testing of New Drug Substances and Products, vol.68, US Food and Drug Administration, DC, USA, 2nd edition, 2003.

20. FDA, ICH-Q1B (R2): Photo-Stability Testing of New Drug Substances and Products, vol.62, US Food and Drug Administration, DC, USA 1997.