Jordan Journal of Pharmaceutical Sciences, Volume 13, No. 2, 2020

Development and Validation of Stability Indicating RP-HPLC Method for Determination of Safinamide Mesylate

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ABSTRACT

A new, simple, specific, accurate and precise RP-HPLC method was developed for determination of Safinamide Mesylate. In the present study, stress testing of Safinamide Mesylate was carried out according to ICH guidelines Q1A (R2). Safinamide Mesylate was subjected to stress conditions of hydrolysis, oxidation, photolysis and neutral decomposition. Successful separation of drug from degradation products formed under stress conditions was achieved on a Hypersil BDS C18 column (250 mm × 4.6 mm, 5.0 µ particle size) using Methanol: Phosphate Buffer pH 6.8 (80:20 % v/v), at a flow rate of 1.0 mL/min and column was maintained at 40˚C. Higher degradation was found to occur in acidic, alkaline, oxidative and photolytic condition. Lesser degradation was observed at thermal conditions. Quantification and linearity was achieved at 226 nm over the concentration range of 40 - 180 µg/mL for Safinamide Mesylate. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness.The developed method is suitable for the routine analysis as well as stability studies.

Keywords: Stability-indicating, HPLC, Safinamide Mesylate, Validation, Stress Testing.

INTRODUCTION

Safinamide mesylate is the methanesulfonic acid form of its active component safinamide, a selective and reversible monoamine oxidase B (MAO-B) inhibitor. It is used for the treatment of Parkinson's disease (PD), safinamide potently modulates dopamine (DA), a substrate of MAO-B, suppressing DA uptake and reversibly binds to MAO-B blocking the function of MAO-B, which lead to the relief of PD symptoms.[1] Besides MAO-B inhibition, safinamide exhibits novel anticonvulsant activities, including sodium channel blockade, calcium channel blockade and glutamate release inhibition.[2]Safinamide mesylate is an orally available derivative from chemical class of · ñ amino amides, with multiple mechanisms of action involving inhibition of MAO-B and Dopamine reuptake used in the treatment of epilepsy and Parkinsonísdisease. Chemically, Safinamide mesylate is, (S)-(+)–2-[4-(3-fluoro-benzyl-oxy-benzyl-amino)propanamide]methane-sulfonate (1:1 salt).[1,2] The Structure is given in Figure 1.

Literature survey reveals that only one enantiomeric chiral chromatographic method[3], a bioassay in fluids human and various animals[4] and a HPLC method[5]has been reported for the estimation of Safinamide mesylate. The aim of the present study is to develop a simple, precise and accurate stability indicating reversed-phase HPLC method[6,7,10,11] for the estimation of Safinamide mesylate in pharmaceutical dosage form as per ICH guidelines.[8,9]

Received on 1/11/2018 and Accepted for Publication on 20/5/2019.

Table 1. % Degradation observed by applying the stress condition

Stress Condition	Acidic	Alkali	Oxidative	Thermal	Photolytic
% Degradation	17.21	27.24	16.84	6.82	13.16

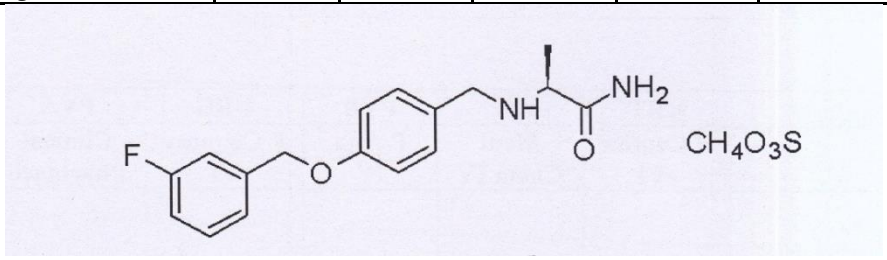

Figure 1: Structure of Safinamide Mesylate

MATERIALS AND METHODS

Materials, Chemicals and Reagents*:*

Safinamide Mesylate (SAF) was supplied as a gift sample by Alkem Pharmaceuticals Ltd, Mumbai, India. All the chemicals used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and HPLC grade water was used for mobile phase preparation.

Instrumentation:

Chromatography was performed under, ambient conditions, with HPLC equipment (Waters Corporation, Milford, USA) consisted comprising quaternary 600 pump, a PDA detector and column C18 (250mm x 4.6 mm i.d., 5 µ particle size) was used. A Rheodyne injector with a 20µl loop was used for the injection of the sample. The HPLC system was equipped with Empower software for data processing. Sartorius microbalance and Equiptronics branded balance and heating oven was used for the weighing and heating purpose while Spinco ultrasonic bath used for degassing purpose.

Method Development:

Mobile Phase Selection*:*

On the basis of literature survey, previous experience and several exploratory efforts, the chromatographic compatibility was achieved using Methanol: Phosphate Buffer pH 6.8 (80:20) as an isocratic elution. This gives the best results as a mobile phase.

Column Selection*:*

Column selection is the most important part in the method development. By applying column chemistry, the most suitable column C18 (250mm x 4.6 mm i.d., 5 µ particle size) was selected.

Detection Wavelength Selection*:*

We screened the standard solution over 190 nm to 400 nm wavelength using the advantage of photo diode array detector. On the basis of peak absorption maxima of analyte and the degradation impurities, the 226 nm was decided as the detection wavelength. This gives the maximum chromatographic compatibility to the method.[6,7]

Mobile Phase Preparation*:*

The mobile phase consist of Methanol: Phosphate Buffer pH 6.8 (80:20) was prepared by dissolving 28.20gm Disodium Hydrogen Phosphate and 11.45 gm Potassium Dihydrogen Phosphate taken in 1000 ml of HPLC grade water and filtered through 0.45µ filter followed by degassing in ultra sonic bath for 20 min. [6,7]

Standard Solution Preparation*:*

A Safinamide mesylate standard solution containing 100µg/ml was prepared in a 100 ml volumetric flask by dissolving 10 mg Safinamide mesylate in 25 ml methanol-water (80:20, v/v) and then diluting to volume with methanol-water (80:20, v/v) to the mark. The sample was filtered through a 0.45 µ nylon syringe filter. The Chromatogram was shown in Figure 2. [6,7]

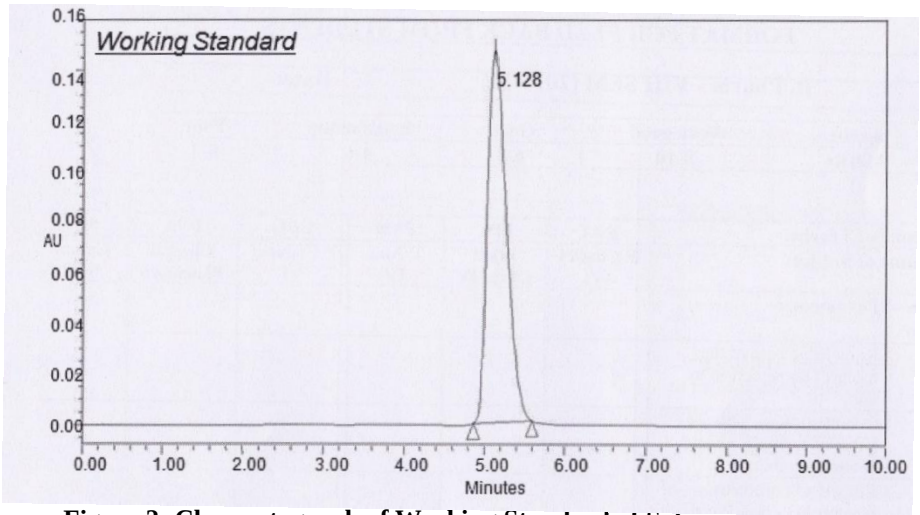

Figure 2: Chromatograph of Working Standard of Safinamide Mesylate

Sample Solution Preparation*:*

Twenty commercially available 50.0mg label claimed tablets of Safinamide mesylate have weighed, and the average weight of a tablet was determined. From these, 2 tablets were weighed and transferred into a 100 ml volumetric flask and added 50 ml methanol-water (80:20, v/v) followed by sonication of minimum 30 min with intermittent shaking. Then the solution has brought back to room temperature and diluted to volume with methanol-water (80:20, v/v). 1.00 ml of above solution was pipette out and transferred into 10 ml volumetric flask followed by diluted to volume with methanol-water (80:20, v/v) diluent. The sample was filtered through a 0.45 µ nylon syringe filter. The final concentration of sample was 100 µg/ml. [6,7]

RESULTS AND DISCUSSION

Method for the determination of Safinamide mesylate in bulk drug as well as in pharmaceutical dosage form is further validated as per ICH Q2 (R1) guideline.[9] To prove this method, Stability Indicating, force degradation study was also being performed and also included. Validation of analytical method was performed using commercially available Safinamide mesylate formulation as well as bulk drug substance equivalent to the formulation.

Specificity*:*

The analyte should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method was quite selective. There was no other interfering peak around the retention time of SAF; also the base line did not show any significant noise.[9]

The evaluation of the specificity of the method was determined against stress (forced) degradation application. Further the specificity of the method toward the drug was established by means of the interference of the degradation products against drug during the forced degradation study.[11]

Forced Degradation Study*:*

The degradation samples were prepared by transferring intact tablets, samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions.[8] After the degradation treatments were completed, the stress content solutions were diluted with diluent to attain about 100 µg/ml concentration.[11] Specific conditions were described as follows.

The method was specific. There was no other interfering peak around the retention time of SAF, also the base line did not show any significant noise. The percent degradations observed by applying the stress conditions are given in Table 1.

A) Acidic Degradation*:* Acidic degradation study was performed by refluxing the drug content in 30 ml of 1 N HCl at about 100° C for 1 hour and after cooling to room temperature it was neutralized with 1 N NaOH solution. Further solution was diluted to achieve concentrations 100 µg/ml with diluent.[8]There was no other interfering peak around the retention time of SAF in acid degradation sample and the degradation achieved up to 17.21%. (Figure 3)

B) Alkali Degradation*:* Alkaline degradation was performed by heating the drug content in 1 N NaOH at around 100°C for 1 hr and then the mixture was neutralized with 1 N HCl. It was further diluted with diluent to achieve 100 µg/ml concentrations.[8]There was no other interfering peak around the retention time of SAF in Alkali degradation sample and the degradation achieved up to 27.24%. (Figure 4)

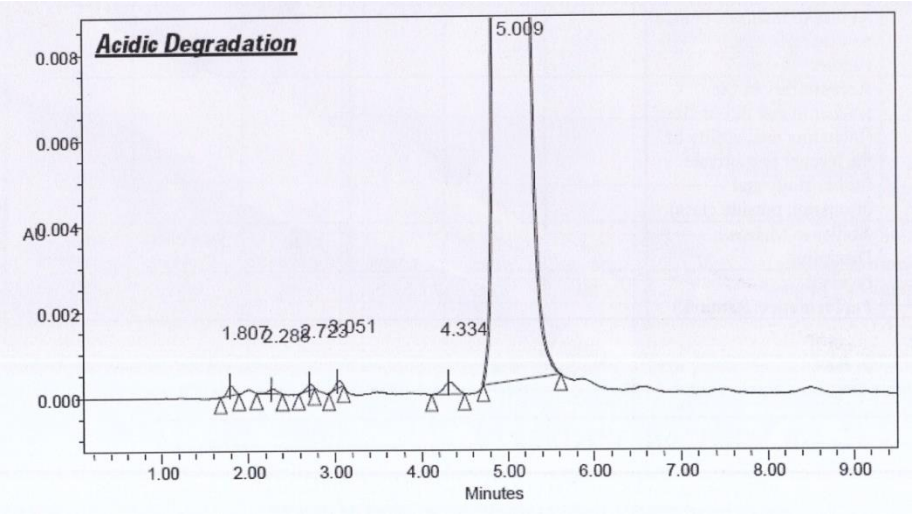

Figure 3: Chromatogram Test preparation (Acidic Degradation)

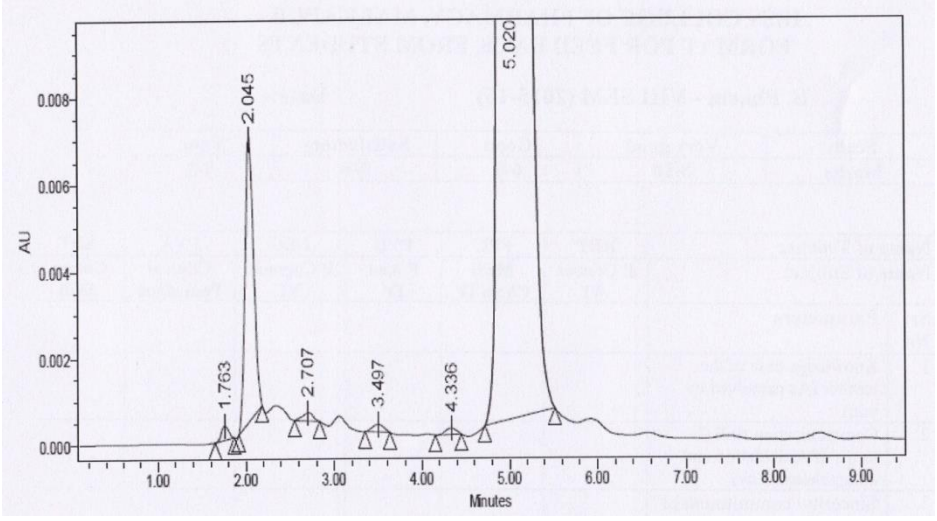

Figure 4: Chromatogram of Alkali Degradation Study

C) Oxidative Degradation Study*:* Oxidative degradation study was performed by refluxing the drug content in 3% v/v H_2O_2 at 100°C for 1 hour then diluted to 100µg/ml with diluent.[8]There was no other interfering peak around the retention time of SAF in oxidative degradation sample and the degradation achieved up to 16.84%. (Figure 5)

D) Thermal Degradation Study*:*

Thermal degradation study was performing by keeping powdered drug content at around 80°C for 72 hours. After that it was allowed to settle at room temperature. This powdered drug was used for the stock solution preparation as per procedure above, then it was diluted to attain 100µg/ml, the same was used in the forced degradation study by confirming against the freshly prepared standard solution. Aim of this experiment was to study the thermal liability of the drug as well as the formulated pharmaceutical dosage form.[8]There was no other interfering peak around the retention time of SAF in Thermal degradation sample and the degradation achieved up to 6.82%. (Figure 6)

E) Photolytic Degradation Study*:* Photolytic degradation study was performed by exposing drug content in sun-light for 72 hour, further it diluted to 100 µg/ml using diluent.[8] There was no other interfering peak around the retention time of SAF in Photolytic degradation sample and the degradation achieved up to 13.16%. (Figure 7)

Any interference was not observed from blank or placebo to the peak of interest, in addition to this peak purity was also within the acceptance criteria proved by the photo diode detector. From the above chromatogram it can be conclude that there is no interference of any degradation product to the peak of interest and impurity has been generated by each stress condition, and hence method is specific.

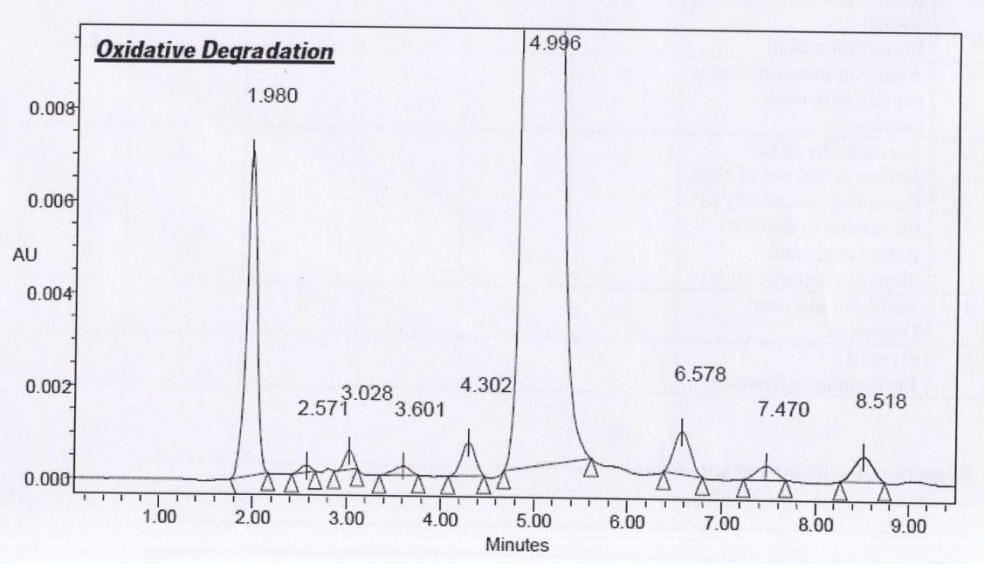

Figure 5: Chromatogram of Oxidative Degradation Study

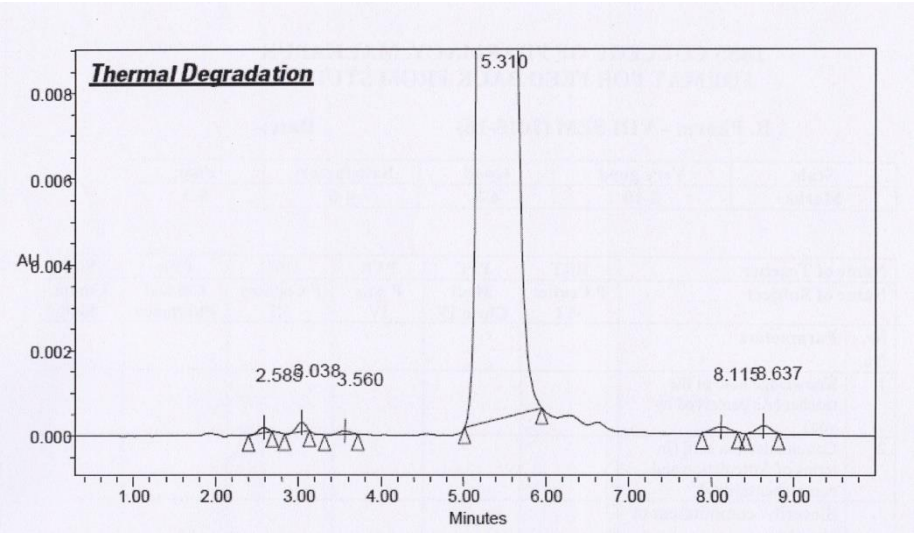

Figure 6: Chromatogram of Thermal Degradation Study

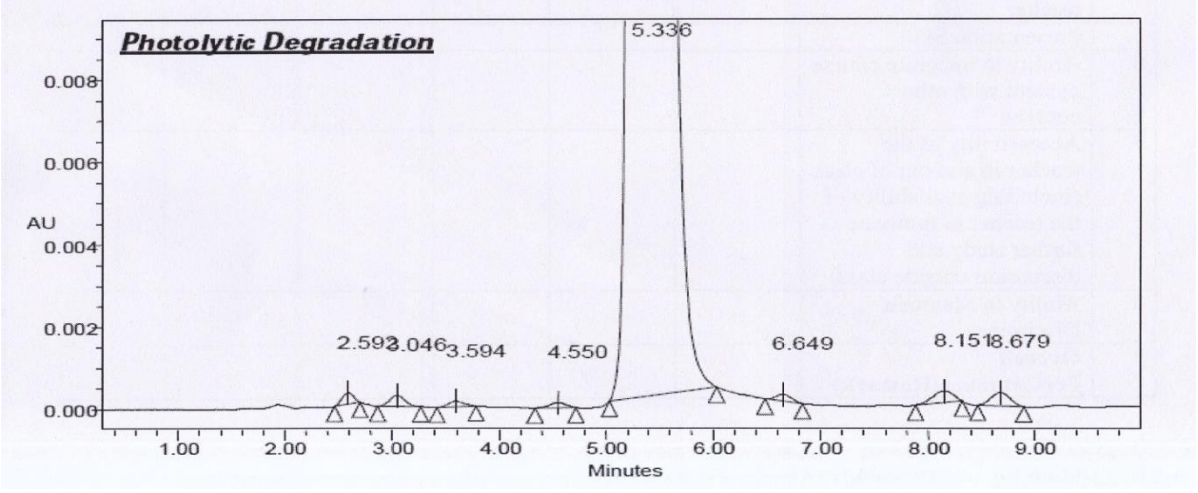

Figure 7: Chromatogram of Photolytic Degradation Study

Linearity and Range*:*

The linearity plot was prepared with 8 concentration levels (40, 60, 80, 100, 120, 140, 160 and 180 µg/ml of Safinamidemesylate). These concentration levels were respectively corresponding to 40, 60, 80, 100, 120, 140, 160 and 180% of standard solution concentration. The Method was found to be linear with Correlation coefficient of the linearity study was found to R^2 = 0.9998 with linear regression equation y = 25174x - 16560, which proves the method is linear over the working range 40 – 180 µg/ml. The peak areas vs. concentration data were evaluated by linear regression analysis[9] (Table 2) .

Table 2.Linearity and Range - Concentration Vs Peak area response data

Linearity Level	Concentration (µg/ml)	Peak Area
1	40	972630
2	60	1516129
3	80	1988284
4	100	2499589
5	120	2998577
6	140	3525684
7	160	4034192
8	180	4485622

Limit of Detection and Limit of Quantitation Study*:*

All the results of LOD and LOQ data were within the acceptance criteria, hence it can be conclude that the LOD and LOQ of the method was 0.15 µg/ml and 0.6 µg/ml respectively which correspond to 0.6% and 0.15% of working concentration. [9]

Precision Study*:*

Precision study was established by evaluating method precision and intermediate precision study as given in Table 3 and Table 4 respectively. Method precision of the analytical method was determined by analyzing six sets of sample solution preparation. Overall the data for the precision study suggest % Assay value for each Test Preparation is between 98 – 102% which is under the acceptance criteria while % RSD of all results are less than 2%. Hence, from all the observation it can conclude that the proposed method is highly precise. [9]

Table 3. Method Precision Data

Samples	Replicates	Peak Area	Mean Area	% Assay
Set 01	Injection 01	2483591	2475255	99.50
	Injection 02	2471654		
	Injection 03	2470520		
Set 02	Injection 01	2484772	2492891.3	101.03
	Injection 02	2496635		
	Injection 03	2497267		
Set 03	Injection 01	2494730	2493557.3	99.72
	Injection 02	2493864		
	Injection 03	2492078		
Set 04	Injection 01	2506943	2508589	98.97
	Injection 02	2502602		
	Injection 03	2516222		
Set 05	Injection 01	2497254	2487344.7	101.09
	Injection 02	2485686		
	Injection 03	2479094		
Set 06	Injection 01	2511534	2494333.3	101.05
	Injection 02	2496617		
	Injection 03	2474849		

Table 4. Intermediate Precision

Intermediate Precision Data				
Samples	**Replicates**	**Peak Area**	**Mean Area**	**% Assay**
Set 01	Injection 01	2390183	2439334	99.08
	Injection 02	2477152		
	Injection 03	2450667		
Set 02	Injection 01	2480432	2461131.67	100.32
	Injection 02	2475165		
	Injection 03	2427798		
Set 03	Injection 01	2454846	2474639	100.80
	Injection 02	2481159		
	Injection 03	2487912		
Set 04	Injection 01	2490325	2470574.33	100.11
	Injection 02	2470837		
	Injection 03	2450561		
Set 05	Injection 01	2470283	2462095	99.79
	Injection 02	2480574		
	Injection 03	2435428		
Set 06	Injection 01	2483442	2463636	99.70
	Injection 02	2450497		
	Injection 03	2456969		

Accuracy Study*:*

This Experiment can be performed by the recovery test. Recovery of the method was evaluated at 3 different concentration levels (Generally corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard to placebo preparation. For each concentration level, 3 sets were prepared and injected in duplicate. [9] The method found to be accurate and its results are shown in Table 5.

Table 5. Summary of Accuracy Data

Accuracy Level	Set No	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery (%)	Average recovery	Std Dev.	% RSD
I (50%)	1	50.13	50.09	99.92	99.9	0.06	0.06
	2	50.10	50.12	100.04			
	3	50.21	50.18	99.94			
II (100%)	1	98.21	98.64	100.44	99.9	0.72	0.72
	2	99.85	100.00	100.15			
	3	100.98	101.03	99.07			
II (150%)	1	149.76	148.66	99.27	99.2	0.11	0.11
	2	149.25	148.04	99.19			
	3	150.27	148.84	99.05			

Robustness Study*:*

Robustness of the method was evaluated by assaying test solutions under slight but deliberate changes in analytical conditions, such as change in flow rate, change in proportions of Methanol : Buffer (82:18 and 78:22,v/v), and change in column-lot. [9] The Method is robust as shown in table 6.

Table 6. Summary of robustness study

Robust Condition	% Assay	Retention time (min.)	System Suitability	
			Theoretical Plates	Asymmetry
Flow Change 0.9 ml/min	101.24	5.91	10824	1.26
Flow Change 1.1 ml/min	99.15	4.57	10773	1.29
MP Proportion Change 82:18	100.64	6.89	10989	1.28
MP Proportion Change 78:22	99.39	4.35	11799	1.23
Column Lot Change	99.84	5.52	11320	1.26

A) Flow Rate change*:* In this experiment the test samples were analyzed at the flow rate of 0.9 ml/min and 1.1 ml/min each and the results were observed in terms of assay value and chromatographic compatibility. The assay value of test preparation was 101.24% and 99.15% at 0.9 and 1.1 ml/min respectively. The chromatograms suggest that the flow rate affects on the retention time of drug but it did not have any impact on the response so the peak area were not change too much hence the assay value remain unaffected.

B) Mobile Phase Proportion Change*:* In this experiment the test samples were analyzed at the mobile phase proportion of 57:43 and 53:47, v/v (Buffer: Methanol) each and the results were observed in terms of assay value and chromatographic compatibility. The assay value of test preparation was 100.64% and 99.39% at Methanol: Buffer (82: 18% v/v) and Methanol: Buffer (78: 22% v/v) respectively. The data and chromatograms suggesting that the Mobile Phase proportion made a impact on the retention time of drug but it did not have any significant change on the intensity so the peak area were not change too much hence the assay value remain unaffected.

C) Column Lot Change*:* In this experiment the test samples were analyzed using different column lot and the results were observed in terms of assay value and chromatographic compatibility. The assay value is for the test preparation was 99.84% and the chromatogram given below suggests that there is no considerable influence of the column lot change on the result of the analysis by this method or on chromatographic suitability of this method. Hence, it can be conclude from this experiment that the method is highly robust in terms of column lot change.

CONCLUSION

A simple stability indicating reverse phase liquid chromatographic method has been developed and validated for the estimation of Safinamide Mesylate in tablet dosage form, the method was found to be specific as there was no interference of any co-eluting impurities after stress degradation study. The proposed method was found to be simple, accurate, precise, sensitive and robust. Hence, it can be used successfully for the routine analysis of Safinamide Mesylate in pharmaceutical dosage forms and for analysis of stability samples obtained during accelerated stability study.

REFERENCES

[1] Marco O., Laura B. and Astrid T,'An expert opinion on safinamide in Parkinson's disease'.*Expert Opinion on Investigational Drugs*; 2008; 17(7), 1115-1125.

[2] Antonio, M, Lorenzo DB, Nunziam, CM, Fabrizio, C, Shevqet, I, Carla, C, Carlo, C, Ruggero, G,'Pharmacokinetics and pharmacodynamics of safinamide, a neuroprotectant with antiparkinsonian and anticonvulsant activity', *Pharmacological Research*, 2004; 50,77–85.

[3] Kai Z., Na X., Xiaowei S., Weina L., Jing M.,Yumin D., 'A validated chiral liquid chromatographic method for the enantiomeric separation of safinamide mesylate, a new anti-Parkinson drug', *Journal of Pharmaceutical and Biomedical Analysis*, 2011, 55,(1), 220–224.

[4] Dal B.L., Mazzucchelli P, Fibbioli, Marzo A, 'Bioassay of safinamide in biological fluids of humans and various animal species', *Arzeneimittel-Forschung*;2006; 56(12), 814-819.

[5] V. K. Redasani, B. J. Mali, A. S. Patil, A. A. Shirkhedkar; Development and validation of RP-HPLC method for determination of safinamide mesylate in bulk and in tablet dosage form, *Analytical Chemistry*; 2013;13(4), 127-130.

[6] V.S. Adhao, R.R. Thenge, Development and Validation of Stability Indicating RP-HPLC Method for Determination of Baclofen, *American Journal ofPharmTech Research*; 2017; 7(2), 544-556.

[7] V.S. Adhao, J. Sharma, M. Thakare; Development and Validation of Stability Indicating RP-HPLC Method for Determination of Ceritinib,*Indonesian Journal of Pharmacy*, 2018; 28(4); 241 – 248.

[8] International Conference on Harmonization (ICH), Harmonized Tripartite Guideline, Stability testing of New Drug Substances and Products, ICH, Q1A (R2), Geneva.

[9] International Conference on Harmonization (ICH), Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Geneva.

[10] LIANG ZOU; LILI SUN; HUI ZHANG; WENKAI HUI;QIAOGEN ZOU; ZHEYING ZHU. Identification, Characterization, and Quantification of Impurities of Safinamide Mesylate: Process-Related Impurities and Degradation Products. *Journal of AOAC International*.2017.,100 (4),1029-1037.

[11] B. P. Shah, S. Jain, K. K. Prajapati and N. Y. Mansuri; Stability Indicating HPLC Method Development: REVIEW; IJPSR, 2012; 3(9), 2978-2988.

تطوير والتحقق من الاستقرار مما يدل على طريقة RP-HPLC لتحديد Safinamide Mesylate

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ملخص

تم تطوير طريقة RP-HPLC جديدة وبسيطة ومحددة ودقيقة لتحديد .Safinamide Mesylate في هذه الدراسة، تم إجراء اختبار الإجهاد لل Safinamide Mesylate وفقًا لإرشادات ICH Q1A (R2). تعرض Safinamide Mesylate لظروف الإجهاد للتحلل المائي والأكسدة والتحلل الضوئي والتحلل المحايد. تحقق الفصل الناجح للدواء من منتجات التحلل التي تشكلت في ظروف الإجهاد على عمود Hypersil BDS C18 حجم الجسيمات (250 مم 4.6 × مم)،حجم (5.0) باستخدام الميثانول :فوسفات العازلة (80:20 6.8 ٪ ت / ت)، في الحفاظ على معدل التدفق من 1.0 مل / دقيقة والعمود عند 40 درجة مئوية، تم العثور على تدهور أعلى تحدث في حالة الحمضية، القلوية، مؤكسد و حال للضوء وقد لوحظ تدهور أقل في الظروف الحرارية، تم تحقيق التقدير الكمي والخطي عند 226 نانومتر على مدى تركيز يتراوح بين 180 - 40 ميكروغرام / مل لسافيناميد ميسيلات، تم التحقق من صحة الطريقة للخصوصية والخطية والدقة والدقة، LOD ، LOQ والمتانة، الطريقة المطورة مناسبة للتحليل الروتيني وكذلك دراسات الاستقرار.

الكلمات الدالة: يدل على الاستقرار ، HPLC ، Safinamide Mesylate ، التحقق من الصحة ، اختبار الإجهاد.

تاريخ استلام البحث2018/11/1 وتاريخ قبوله للنشر 2019/5/20.