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Development and validation of RP-HPLC method for determination of safinamide mesylate in bulk and in tablet dosage form

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ABSTRACT

A rapid, highly sensitive high performance liquid chromatographic method has been developed for the determination of Safinamide mesylate in bulk drug and in tablets. Safinamide mesylate was eluted from a NEOSPHER RP C_{18} reversed phase column with a mobile phase consisting of methanol and water (80:20, *v/v*) at a flow rate of 1 mL/min with UV detection at 226 nm. The retention time for Safinamide mesylate was 5.2 min. The linear response (r^2 = 0.9998) was observed in the range of 5 - 30 µg/mL with limits of detection (LOD) and quantification (LOQ) being 0.27 and 0.83 µg, respectively. The method shows good recoveries and intra and inter-day relative standard deviations were less than 1.0%. Validation parameters as specificity, accuracy, ruggedness and robustness were also determined. The proposed method provides accurate and precise quality control tool for routine analysis of Safinamide mesylate in bulk and in tablet dosage form.

KEYWORDS

Safinamide mesylate;
RP-HPLC;
Validation.

INTRODUCTION

Safinamide mesylate (SAF) is an orally available derivative from chemical class of α – amino amides, with multiple mechanisms of action involving inhibition of MAO-B and Dopamine reuptake used in the treatment of epilepsy and Parkinson's disease. Chemically, Safinamide mesylate is, (*S*)-(+)-2-[4-(3-fluorobenzyloxybenzylamino) propanamide] methane-sulfonate (1:1 salt). The chemical structure is shown in Figure 1.[1, 2]

Literature survey reveals a validated chiral liquid chromatographic method for the enantiomeric separation of safinamide mesylate[3] and bioassay of safinamide mesylate in biological fluids of humans and various animal species[4].

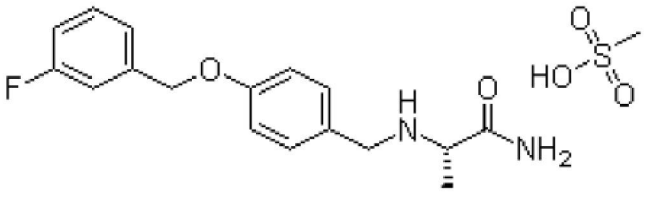

Figure 1 : Chemical structure of safinamide mesylate (SAF)

Except this, only single HPTLC method[5] was available for estimation of SAF as indicated by detail literature survey. This encourages us to undertake this work, so that quantitative estimation of SAF can be done and hence can be used for routine analysis of bulk

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and formulation as well.

The present study describes the development and validation of a simple, specific, sensitive, accurate and precise RP - HPLC method for the determination of SAF in tablet dosage form. The proposed method is optimized and validated according to ICH guidelines[6]

EXPERIMENTAL

Materials 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 double distilled R.O water was used for mobile phase preparation.

Equipment

The development and validation of the assay was performed on a Younglin ACME - 9000 HPLC system (South Korean) provided with a SP930 D Dual Piston Reciprocating Pump, UV VIS (UV 730 D) spectrophotometric detector with the variable wavelength and Autochro-3000 Data processor. SHIMADZU AUX – 120 Weighing Balance was used for all weighings.

Chromatographic conditions

A stationary phase with C_{18} bonded phase i.e. NEOSPHER RP C_{18} (250 mm x 4.6 mm I.D.) with particle size 5 µm was selected with mobile phase Methanol: water in proportion 80:20, v/v. The mobile phase was degassed by sonication. 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 5.2 min. (Figure 2)

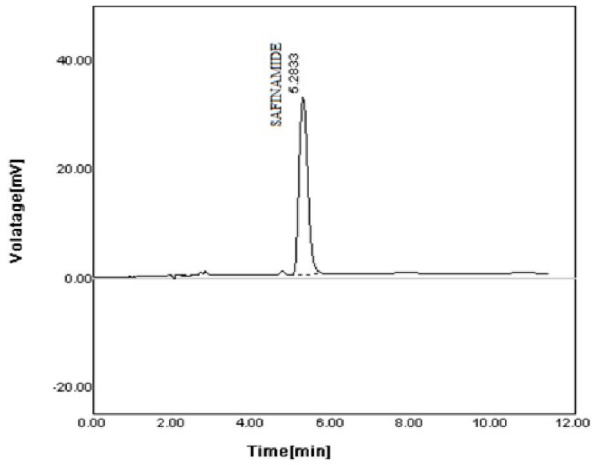

Figure 2. Chromatogram of standard Safinamide Mesylate in mobile phase of methanol and water (80:20, v/v) at a flow rate of 1 mL/min with UV detection at 226 nm

Preparation of stock and standard solution

Stock solution was prepared by dissolving 10 mg of SAF in 10 mL mobile phase to yield concentration of 1000 µg/mL. This solution was diluted with mobile phase as needed to prepare different standard solutions.

RESULTS AND DISCUSSION

Linearity studies

From stock solution, aliquots of 0.5, 1, 1.5, 2, 2.5 and 3 mL were taken in 10 mL volumetric flasks and diluted up to the mark with the mobile phase, such that the final concentration obtained in the range 5 – 30 µg/mL. Calibration curve was constructed by plotting the peak area *vs* the drug concentration. The calibration curve is shown in Figure 3.

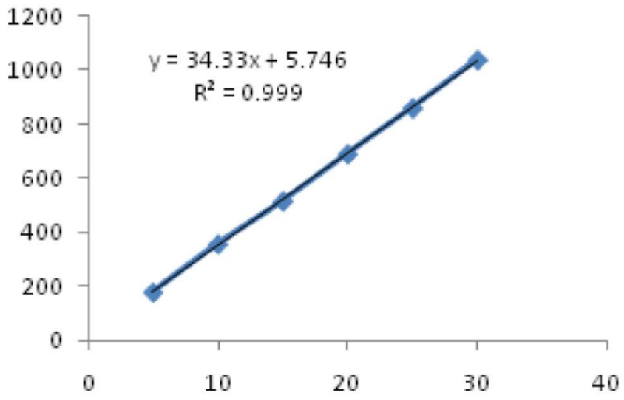

Figure 3: Calibration curve of Safinamide Mesylate

Application of proposed method to tablet formulations

To determine the content of SAF in conventional tablets (Label claim 50 mg SAF per tablet); twenty tablets were weighed, their mean weight was determined, they were finely powered and powder equivalent 10 mg SAF was transferred into a 100 mL volumetric flask and diluted up to 100 mL with methanol, sonicated for 20 min. The resulting solution was filtered, using 0.45 µm filter (Millifilter, Milford, MA) for separating excipients. The solution was further diluted to get concentration of 15 µg/mL was subjected to proposed method and amount of SAF was determined.

(TABLE 1)

TABLE 1: Analysis of tablet formulation (Average weight = 0.180 gm)

Amount taken [µg/mL]	Amount found [µg/mL] Mean ± S.D.	% Amount found Mean ± S.D.	%R.S.D.*
15	15.10 ± 0.11	100.67 ± 0.78	0.77

*n=3

Validation of test procedure:

The objective of validation of an analytical procedure is to demonstrate that it is adequate for its intended purpose. To meet the pharmaceutical regulatory guidelines i.e., ICH guidelines[6] a number of parameters must be investigated in order to validate analytical methods such as accuracy, precision, sensitivity, specificity, ruggedness and robustness study.

Accuracy

It was done by recovery study using standard addition method at 80, 100 and 120 % level; known amount of standard SAF was added to pre-analyzed sample (10 µg/mL of SAF) and subjected them to the proposed HPLC method. Results are shown in TABLE 2.

TABLE 2 : Results of recovery studies

Initial amount of drug [µg/mL]	Excess drug added to the analyte [%]	Amount added [µg/mL]	% Recovery	% R.S.D.*
10	0	0	100.01	0.61
10	80	8	100.17	0.91
10	100	10	100.07	0.73
10	120	12	99.95	0.58

*n=3

Intra – day and inter – day precision

Intra – day precision was determined by analyzing, the three different concentrations 10, 15 and 20 µg/mL of SAF, for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three different days. These result shows reproducibility of the assay. The % R.S.D. values are showed in TABLE 3, i.e. less than 2 and low standard deviation, so this indicates that, method is precise for the determination of Safinamide mesylate.

TABLE 3 : Results of precision studies (Intra-day and Inter-day)

Conc.(µg/mL)	Intra-day Amount found (%)		Inter-day Amount found (%)	
	Mean ± S.D.*	% R.S.D.	Mean ± S.D.*	% R.S.D.
10	100.31 ± 0.26	0.26	100.59 ± 0.87	0.87
15	100.04 ± 0.14	0.14	100.17 ± 0.44	0.44
20	100.41 ± 0.15	0.15	100.51 ± 0.063	0.063

*n=3

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD and LOQ were found to be 0.27 and 0.83 µg/mL for SAF, respectively.

Specificity and Selectivity

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.

Ruggedness

From stock solution, sample solution of SAF 15 µg/mL was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same

TABLE 4 : Results of ruggedness study

Analyst	% Amount found (Mean ± S.D.)	% R.S.D.*
I	100.48 ± 0.57	0.57
II	100.89 ± 0.63	0.63

*n=3

concentration solutions, three times. The results are reported in TABLE 4.

Robustness

To evaluate robustness, few parameters were deliberately varied like variation of flow rate, changing the pH of the solution, changing mobile phase composition, using 15 µg/mL solution of SAF. Results are shown in TABLE 5.

TABLE 5 : Results of robustness studies

Sr. no.	Parameter	S.D. of peak area	% R.S.D.*
1	**Mobile phase composition**		
	Methanol : Water (85:15)	1.36	0.35
	Methanol : Water (75:25)	2.54	0.64
2	**Changing flow rate**		
	a) 0.8 mL/min	1.78	1.54
	b) 1.2 mL/min	0.41	0.78
3	**Changing pH of mobile phase**		
	a) 6.5	1.55	0.54
	b) 7.5	0.68	0.41

***n=3**

CONCLUSION

A new reversed-phase high performance liquid chromatographic method for the determination of safinamide in bulk and in pharmaceutical formulation was developed. The developed method is very simple and results obtained confirm suitable accuracy, specificity, precision, ruggedness and robustness. Therefore, the developed RP HPLC method was proved to be suitable for the safinamide determination in tablets.

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