Research Article

Development and Validation of HPTLC Method for Estimation of Safinamide Mesylate in Bulk and in Tablet Dosage Form

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1. Introduction

Safinamide (SAF) is an orally available derivative from chemical class of α-amino amides, with multiple mechanisms of action involving inhibition of mono-amino oxidase B and dopamine reuptake used in the treatment of epilepsy and parkinson’s disease. Chemically, Safinamide is (S)-(+)-2-[4-(3-fluorobenzyloxybenzylamino) propanamide] methane sulfonate (1 : 1 salt) (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 in biological fluids of humans and various animal species [4].

Except this, so far no analytical method was available for estimation of SAF as indicated by detail literature survey. The therapeutic effectiveness and less methods available for its estimation encourage us to undertake this work, so that quantitative estimation of SAF can be done and hence can be used for routine analysis of bulk and formulation as well.

The present study describes the development and validation of a simple, specific, sensitive, accurate precise, and economic HPTLC method for determination of SAF in tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [5, 6].

2. Experimental

2.1. Chemicals and Reagents. Safinamide mesylate was kindly gifted from Alkem Pharmaceuticals, Mumbai (Maharashtra), India. Safinamide tablets were obtained from commercial sources within their shelf life period. All the reagents and solvents used were of analytical reagent (AR) grade. Solvents used like toluene, methanol, and triethylamine were of AR grade and obtained from Merck Chemicals.

2.2. Instrumentation and Chromatographic Conditions. The samples were spotted in the form of bands of width of 6 mm with a Camag 100 μL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminum plate 60 F254 (20 cm × 10 cm with 0.2 mm thickness) supplied by Anchorm technologists (Mumbai) using a CAMAG Linomat 5 sample applicator (Switzerland). A constant application rate of 200 nL sec⁻¹ was employed, and the space between two bands
was 15.4 mm. The slit dimension was kept 6 mm × 0.45 mm. The mobile phase consisted of toluene : methanol : triethylamine (4 : 1 : 0.5 v/v) was selected, which gave sharp and symmetrical peak with RF value of 0.54 at 226 nm (Figure 2).

2.5. Application of Proposed Method to Tablet Formulation. To determine the concentration of SAF in tablets (Label claim: 50 mg per tablet), the contents of 20 tablets were weighed, their mean weight determined, and were finely powdered. The powder equivalent to 10 mg of SAF was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 20 min and the volume was made up to 10 mL. The resulting solution was filtered using 0.41 μm filter (Millifilter, Milford, MA). The above solution was applied on TLC plate (1000 ng per spot) followed by development and scanning as described in above chromatographic conditions (Table 1).

3. 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, limit of detection (LOD), and limit of quantification (LOQ).

3.1. Linearity (Calibration Curve). For linearity study, aliquots of 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 μL of SAF from standard stock solution was applied on TLC plate to obtain the concentration of 400, 800, 1200, 1600, 2000, and 2400 ng/spot. TLC plates were developed under the above-established conditions. Area under peak was recorded and plotted against concentration (Figure 3).

3.2. Accuracy. The preanalyzed samples of concentration 1000 ng μL⁻¹ were over spotted with excess 80, 100, and 120% of standard drug. The total concentrations of the drug was determined (n = 3), to check for the recovery of the drug at different levels in formulation.

3.3. Specificity. The specificity of the method was ascertained by analyzing standard drug and formulation. The spot for SAF in formulation was confirmed by comparing the RF values and spectra of the spot with that of standard. The peak

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### Table 1: Analysis of Tablet formulation (Label claim: 50 mg per tablet).

<table>
<thead>
<tr>
<th>Concentration (ng μL⁻¹)</th>
<th>Amount found (ng)</th>
<th>Amount found (%)</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1008.8</td>
<td>100.8</td>
<td>99.97</td>
</tr>
<tr>
<td>999.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>995.63</td>
<td></td>
<td></td>
<td>99.56</td>
</tr>
<tr>
<td>1006.7</td>
<td></td>
<td></td>
<td>100.6</td>
</tr>
<tr>
<td>1011.3</td>
<td></td>
<td></td>
<td>101.1</td>
</tr>
<tr>
<td>994.14</td>
<td></td>
<td></td>
<td>99.41</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>1002.7 ± 7.22</td>
<td>100.2 ± 0.72</td>
<td>0.72</td>
</tr>
</tbody>
</table>

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2.4. Optimization of HPTLC Method. Initially, different ratios of methanol, chloroform, n-propanol, and toluene were tried, but tailing of spots was observed. Finally, the mobile phase toluene : methanol : triethylamine (4 : 1 : 0.5% v/v) gives good resolution, sharp and symmetrical peak with RF value of 0.54 at 226 nm (Figure 2).
3.4. **Precision.** Repeatability was determined by spotting 1200 ng per spot of SAF. Precision of the method was assessed by spotting 800, 1200, and 1600 ng per spot of SAF on three different times within the same day (intraday) and on three different days (interday).

3.5. **Robustness.** Robustness of the method was performed by spotting 1200 ng of drug making small deliberate changes in various chromatographic conditions. Mobile phases having different composition of toluene: methanol: TEA (3.8:1.2:0.5 and 4.3:0.7:0.2 v/v) were tried, and chromatograms were run. The volume of mobile phase, temperature, and relative humidity was varied in the range of ±5%.

### Table 2: Results of recovery studies.

<table>
<thead>
<tr>
<th>Initial amount of drug (ng μL⁻¹)</th>
<th>% of standard drug added</th>
<th>% Recovery*</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>80</td>
<td>99.44</td>
<td>0.31</td>
</tr>
<tr>
<td>1000</td>
<td>100</td>
<td>99.88</td>
<td>0.33</td>
</tr>
<tr>
<td>1000</td>
<td>120</td>
<td>99.86</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*Mean of three estimations at each level.

The plates were prewashed by methanol and activated at 60 ± 5°C for 2, 5, and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied as 0, 20, and 40 min.

3.6. **Ruggedness.** Ruggedness of the method was performed by spotting 1200 ng of drug by two different analysts maintaining same experimental and environmental conditions.

3.7. **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 400, 480, 560, 640, 720, and 800 ng μL⁻¹ 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 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.

### 4. Results and Discussion

4.1. **HPTLC Method Development.** The TLC procedure was optimized with a view to develop a method for determination of SAF. Optimization of mobile phase with toluene: methanol: triethylamine (4:1:0.5 v/v) gives sharp and symmetrical peak having RF value of 0.54 ± 0.2. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and presaturation of TLC chamber with mobile phase for 15 min ensures good reproducibility and peak shape.

4.2. **Validation**

4.2.1. **Linearity.** The linear regression data for the calibration curves showed good linear relationship over the concentration range 400–1200 ng μL⁻¹. Linear regression equation was found to be \( Y = 2.474X + 44.52 \) \( (r^2 = 0.999) \).

4.2.2. **Accuracy.** The proposed method when used for extraction and subsequent estimation of drug from tablet dosage form after over spotting with 80, 100, and 120% of additional drug; mean recovery is within acceptable limits, indicating the method is accurate and afforded recovery of 99.44–99.88% (Table 2).

4.2.3. **Specificity.** The peak purity of SAF was assessed by comparing the spectra at three different levels, that is, peak start (S), peak apex (M), and peak end (E) positions of
4.2.4. **Precision.** The precision of the developed HPTLC method was expressed in terms of % RSD. The results depicted revealed high precision of the method (Table 3).

4.2.5. **Robustness.** The standard deviation of peak areas was calculated for each parameter, and % RSD was found to be less than 2% (Table 4).

4.2.6. **Ruggedness.** The % RSD was found to be less than 2% indicating the method was rugged when estimation was done by two different analysts, Table 5.

4.2.7. **LOD and LOQ.** Detection limit and quantification limit for SAF were found to be 13.09 ng and 39.67 ng, respectively. This indicates adequate sensitivity of the method as it can be validated in less quantity of drug, and hence the method proves to be economic.

5. **Conclusion**

The literature survey promoted us to develop HPTLC method on SAF as no analytical method was reported for it. The HPTLC method was developed and validated as per ICH guidelines, and the method was found to be simple, precise, accurate reproducible, and economic, thus can be used for determination of SAF in tablets. Moreover, proposed method also indicates no interference of excipients when applied to tablet dosage form. Future plan includes development of Safinamide mesylate in available combinations along with stability-indicating and forced degradation-studies.

**Acknowledgments**

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**References**


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