Development of validated HPTLC method for densitometric estimation of lacosamide in bulk drug and tablet dosage form

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ABSTRACT

A simple, selective, precise, and reproducible high-performance thin-layer chromatographic (HPTLC) method for the analysis of lacosamide in bulk drug and tablet dosage form was developed. The study was performed on pre-coated (silica gel aluminium plates 60 F-254). The chromatograms of samples were developed in twin trough glass chamber pre-saturated with mobile phase comprised of toluene: methanol: ammonia (30%) (7.5:2:0.5 v/v/v) at room temperature (25 ± 2°C). The densitometric analysis was carried out in absorbance mode at 258 nm. The optimized mobile phase showed a compact spot of lacosamide (Rf = 0.24 ± 0.02). The linear regression analysis data for the calibration plots showed good linearity (r^2 = 0.995) with respect to peak area in the range of 200–2000 ng spot−1. The method was validated as per International Conference on Harmonization (ICH) guidelines. The limits of detection and quantification (50 and 200 ng spot−1 respectively) were also established. The proposed method has shown the excellent recovery (99.65–101.02%) which supports the suitability of the method for the analysis. Statistical analysis of the obtained data showed the selectivity of the proposed method for lacosamide estimation as a bulk drug and tablet dosage form.

Key words: Lacosamide, HPTLC, Method development, Densitometric determination, Validation.

INTRODUCTION

Lacosamide (Fig.1) is chemically known as (2R)-N-benzyl-2-acetamido-3- methoxypropanamide. Its chemical formula is C13H18N2O3 with a molecular weight 250.294 g mol⁻¹. It is white to light yellow powder, sparingly soluble in water and slightly soluble in acetonitrile and ethanol. Lacosamide is an anticonvulsant drug used in the treatment of partial-onset seizures [1-2].

Figure 1: Chemical structure of lacosamide
Lacosamide is a functionalized amino acid that has activity in the maximal electroshock seizure test and is indicated for the adjunctive treatment of partial-onset seizures and diabetic neuropathic pain. Recent studies indicate that lacosamide only affects those neurons which are depolarized or active for long periods of time, typical of neurons at the focus of an epileptic seizure as opposed to other antiepileptic drugs such as carbamazepine or lamotrigine which slow the recovery from inactivation and reduce the ability of neurons to fire action potentials [3-4].


However, our aim was to develop a validated [13, 14] HPTLC method for estimation of lacosamide in bulk and tablet dosage forms which has lower limit of detection and quantification than the reported method by Kamdar et al. (2012) [12].

**MATERIALS AND METHODS**

2.1. Chemicals and reagents
Lacosamide pure drug was a gift sample procured from Glenmark Pharmaceuticals Ltd., Mumbai, Maharashtra, India. All the solvents and reagents used for analysis were of analytical grade (Merck, Mumbai, India).

2.2. HPTLC instrumentation
A Camag HPTLC system equipped with Linomat V applicator (Switzerland), TLC Scanner III and integrated software Win-Cats (V 3.15, Camag) was used for the analysis. The standard and the sample solutions were spotted in the form of bands of width 6 mm with a Camag 100 μL sample (Hamilton, Bonaduz, Switzerland) syringe, on silica gel pre-coated aluminum plate 60F-254 plates (20 × 10 cm) with 250 μm thickness; (E. Merck, Darmstadt, Germany), supplied by Anchorm technologist, Mumbai. A constant application rate of 150 nL s$^{-1}$ and space between bands (5 mm) were employed. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. The slit dimension was kept at 5 mm × 0.45 mm, data resolution of 100 μm step$^{-1}$ and the scanning speed was 20 mm s$^{-1}$. The monochromatic band width was set at 258 nm, each track was scanned three times and baseline correction was used.

The mobile phase consisted of toluene: methanol: ammonia (30%) (7.5: 2: 0.5 v/v/v) and 10 mL of mobile phase was used per chromatographic run. Linear ascending development was carried out in a (20 cm × 10 cm) twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was (20 min) at room temperature (25°C ± 2) at relative humidity of 60% ± 5. Each chromatogram was developed over a distance of 80 mm. Following the development the TLC plates were dried in a stream of air with the help of hair dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed at 258 nm. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis.

2.3. HPTLC method and chromatographic conditions
2.3.1. Preparation of standard stock and working standard solutions
For preparation of standard stock and working standard solutions, an accurately weighed quantity of lacosamide (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol. Then mixture was sonicated for 20 min. Volume was made up to the mark with methanol to give the concentration of (1000 ng spot$^{-1}$).

2.3.2. Prewashing of plates
Densitometric estimation was carried out on (20 cm × 10 cm) pre-coated silica gel 60 F-254 plates from E. Merck. The plates were pre-washed with methanol, dried and activated for 15 min at 110°C.

2.3.3. Selection of solvent
Methanol was selected as a solvent for preparing drug solutions.
2.3.4. Selection of stationary phase
Identification and separation of lacosamide was carried out on (20 cm × 10 cm), pre-coated silica gel aluminium plates 60 F-254 (250 µm thickness E. Merck, Darmstadt, Germany).

2.3.5. Sample application
The standard and working standard solutions of lacosamide were spotted on pre-coated TLC plates in the form of narrow bands of length 5 mm at 10 mm from the bottom and left margin and 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL s⁻¹.

2.3.6. Selection of wavelength
Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis at 258 nm using methanol as a blank solution. The selection of wavelength display is shown in (Fig. 2).

![Figure 2: Spectrum for selection of wavelength (258 nm)](image)

2.3.7. Optimization of the mobile phase
Various solvent systems like mixture of (a) toluene: methanol (7:3 v/v) (b) triethylamine: methanol (6: 3 v/v) (c) toluene: chloroform: methanol (1: 5: 3 v/v/v) and (d) toluene: ethyl acetate: methanol (0.5: 4: 2 v/v/v) were tried to separate and resolve spot of lacosamide from its impurities and other excipients of formulation. The mixture of toluene: methanol (7.5: 2 v/v/v) resolved lacosamide but there was tailing in the peaks. To improve peak symmetry, 30% ammonia was added. Finally, the mixture of toluene: methanol: ammonia (30%) (7.5: 2: 0.5 v/v/v) showed well resolved peak with better peak shape. The drug was resolved with (Rₑ = 0.24 ± 0.02). Pre-saturation of TLC chamber with mobile phase for 20 min assured better reproducibility in migration of lacosamide and better resolution.

2.4. Method validation
The developed HPTLC method was validated as per the ICH guidelines Q2 (R1) for linearity, precision, repeatability, accuracy, specificity, robustness, limit of detection (LOD), limit of quantification (LOQ).

2.4.1. Linearity (Calibration curve)
A stock solution (1000 ng spot⁻¹) of lacosamide was prepared by dissolving it in methanol. 10 different concentrations of lacosamide (200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 ng spot⁻¹) were applied on the TLC plate. The data obtained were treated by least-square regression analysis method.

The linearity range of lacosamide was obtained by plotting the peak area of lacosamide against its varied concentrations over a range (200–2000 ng spot⁻¹).
2.4.2. Precision
The intra and inter-day variations were determined using three different concentration levels 200, 300, and 400 ng spot$^{-1}$ of lacosamide ($n = 3$). The precision of the developed method was evaluated by performing repeatability of the sample application and peak area measurement in six replicates of the same spot. The results are expressed in terms of percent relative standard deviation (% RSD) and standard error (SE).

2.4.3. Repeatability
It is also termed as intra-assay precision. Repeatability of sample application was assessed by spotting (1200 ng spot$^{-1}$) of standard drug solution six times on TLC plate at different times on same day by sample applicator, followed by development of plate and recording of the peak areas for six spots.

2.4.3. Recovery and specificity studies
Recovery studies were carried out to determine accuracy of the developed method at 80%, 100%, and 120% levels. It was done by mixing known quantity of standard drug (1000 ng spot$^{-1}$) with the sample formulation and contents were analyzed by the proposed method. The % recovery and % RSD were calculated respectively.

The specificity of the method was ascertained by analyzing the $R_f$ values and spectra pattern of reference marker and drug samples. The marketed formulation, Lacoset 100 mg (Sun Pharma laboratories Ltd. Sikkim, India) was sonicated (10 mg in 5 mL methanol) for 20 min. The volume was made up to 10 mL by adding methanol. The resulting solution was centrifuged, and the supernatant was filtered. The amount of lacosamide was determined by developing the chromatogram (1000 ng spot$^{-1}$) in triplicate by maintaining the chromatographic conditions. The spot for lacosamide in formulation was confirmed by comparing the $R_f$ and densitogram of the spot with that of standard.

2.4.5. Robustness
In this study, small changes in the composition and volume of mobile phase and development chamber saturation time were made and their effects on the results were examined. The study was done in triplicate at a concentration 1200 ng spot$^{-1}$ of lacosamide. The results of peak area are expressed as % RSD and SE.

2.4.6. Limit of detection (LOD) and limit of quantification (LOQ)
LOD and LOQ were experimentally determined by visual detection method recommended in ICH guidelines. To estimate the LOD and LOQ blank methanol was spotted six times. Spotting for LOD was done by taking different concentrations as 50, 100, 150, 200, and 250 ng spot$^{-1}$ and the values were considered with a signal-to-noise ratio of 3:1 and 10:1 respectively.

RESULTS AND DISCUSSION

3.1. Development of TLC procedure
The TLC procedure was developed and optimized with a view to quantify the lacosamide content in standard and in test samples. The mobile phase toluene: methanol: 30% ammonia (7.5:2:0.5 v/v/v) was optimized and selected by trial and error method on the basis of resolution with a sharp and well defined peak at $R_f = 0.24\pm0.02$ (Fig. 3).
3.2. Calibration curve

The developed HPTLC method for estimation of lacosamide showed a correlation coefficient ($r^2 = 0.995$) with SD 11.23 and intercept 2.566 in the concentration range of 200–2000 ng spot$^{-1}$ (Table 1) with respect to the peak area. (Fig. 4) displays the calibration curve of lacosamide at 258 nm.

The linearity of calibration graphs and adherence of the system to Beer’s law was validated by correlation coefficient. No significant difference was observed in the slopes and standard curves (ANOVA, $p<0.05$).

$$y = 0.673x + 2.5667 \quad R^2 = 0.9952$$

![Calibration curve of lacosamide](image)

Table 1 Summary of linear regression and validation data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HPTLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (ng spot$^{-1}$)</td>
<td>200-2000</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.995</td>
</tr>
<tr>
<td>Intra-day precision (n=3)</td>
<td>10.82 ± 0.94, 3.60</td>
</tr>
<tr>
<td>Inter-day precision (n=3)</td>
<td>10.33 ± 0.90, 3.44</td>
</tr>
<tr>
<td>Limit of detection (ng spot$^{-1}$)</td>
<td>50</td>
</tr>
<tr>
<td>Limit of quantification (ng spot$^{-1}$)</td>
<td>200</td>
</tr>
<tr>
<td>Recovery (n=3) (%)</td>
<td>100.32%</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
</tr>
</tbody>
</table>

3.3. Validation of the method

3.3.1. Precision

The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria which indicated that the method was precise. In precision studies on intra and inter-days, the resultant peak area for lacosamide determined at three different concentration levels (200, 300, 400 ng spot$^{-1}$ of lacosamide) showed % RSD (<1.5%) for inter- and intra-day variations which suggested an excellent precision of the method (Table 2).

Table 2 Precision study (n= 3)

<table>
<thead>
<tr>
<th>Precision</th>
<th>Conc. (ng spot$^{-1}$)</th>
<th>$R_f$</th>
<th>S D</th>
<th>S E</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day</td>
<td>200</td>
<td>0.24</td>
<td>10.82</td>
<td>3.60</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.24</td>
<td>10.28</td>
<td>3.42</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.24</td>
<td>11.21</td>
<td>3.7</td>
<td>0.81</td>
</tr>
<tr>
<td>Inter-day</td>
<td>200</td>
<td>0.24</td>
<td>10.33</td>
<td>3.44</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.24</td>
<td>11.06</td>
<td>3.68</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.24</td>
<td>10.24</td>
<td>3.41</td>
<td>0.74</td>
</tr>
</tbody>
</table>

SD = standard deviation; RSD = relative standard deviation; SE = standard error.
3.3.2. Repeatability
The % RSD for repeatability of the drug was found to be (<2). The measurement of peak areas at three different concentration levels showed low value of % R.S.D. (<2) and low value of S.E. (Table 3). Hence the proposed method for estimation is proved to be repeatable in nature.

Table 3 Repeatability study (n=6)

<table>
<thead>
<tr>
<th>Concentration (ng spot⁻¹)</th>
<th>Area</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200</td>
<td>577.8</td>
<td>4.29</td>
<td>0.74</td>
</tr>
<tr>
<td>1200</td>
<td>591.62</td>
<td>5.03</td>
<td>0.84</td>
</tr>
<tr>
<td>1200</td>
<td>598.08</td>
<td>6.42</td>
<td>1.06</td>
</tr>
<tr>
<td>1200</td>
<td>598.47</td>
<td>6.05</td>
<td>1.00</td>
</tr>
<tr>
<td>1200</td>
<td>597.72</td>
<td>5.02</td>
<td>0.82</td>
</tr>
<tr>
<td>1200</td>
<td>613.25</td>
<td>6.87</td>
<td>1.11</td>
</tr>
</tbody>
</table>

SD = standard deviation; RSD = relative standard deviation.

3.3.3. Recovery and specificity studies
Results of the recovery study showed high efficiency of lacosamide from the samples. The proposed method afforded recovery in the range of 99.65–101.02 % (Table 4). This confirms that the proposed method can be used for the determination of lacosamide in formulations at different concentration levels.

The peak purity of lacosamide was assessed by comparing their respective densitograms at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = (0.20 -0.24) and r (middle, end) = (0.24 -0.29). Good correlation was obtained between standard and sample densitograms of lacosamide. The chromatogram is shown in (Fig.5).

Table 4 Recovery study (n=3)

<table>
<thead>
<tr>
<th>Levels (%)</th>
<th>Conc. Added (ng spot⁻¹)</th>
<th>Conc. found (ng spot⁻¹)</th>
<th>SD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>1000</td>
<td>996.58</td>
<td>4.13</td>
<td>99.65</td>
</tr>
<tr>
<td>100</td>
<td>1200</td>
<td>1203.61</td>
<td>5.06</td>
<td>100.30</td>
</tr>
<tr>
<td>120</td>
<td>1300</td>
<td>1313.37</td>
<td>5.34</td>
<td>101.02</td>
</tr>
</tbody>
</table>

SD = standard deviation

Figure 5: Chromatogram of tablet (Lacoset 100 mg) of lacosamide
3.3.4. Robustness of method

The % R.S.D. and S E of the peak areas was calculated for change in mobile phase composition, mobile phase volume, temperature, time from spotting to chromatography and time from chromatography to scanning in triplicate at concentration level of (1200 ng spot⁻¹) of lacosamide. The deviation obtained by deliberate changes in various parameters % R.S.D (<2) (Table 5) which indicated that the developed HPTLC method was robust.

Table 5 Results of robustness testing

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SD</th>
<th>SE</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase composition (±) 0.1mL</td>
<td>4.21</td>
<td>2.43</td>
<td>0.72</td>
</tr>
<tr>
<td>Amount of mobile phase (±) 0.1mL</td>
<td>6.03</td>
<td>3.48</td>
<td>0.84</td>
</tr>
<tr>
<td>Temperature (±) 5ºC</td>
<td>5.42</td>
<td>3.12</td>
<td>0.82</td>
</tr>
<tr>
<td>Time from spotting to chromatography (±) 5 min</td>
<td>5.09</td>
<td>2.93</td>
<td>0.86</td>
</tr>
<tr>
<td>Time from chromatography to scanning (±) 5 min</td>
<td>6.97</td>
<td>4.02</td>
<td>1.14</td>
</tr>
</tbody>
</table>

SD = standard deviation; RSD = relative standard deviation; SE=standard error.

3.3.5. LOD and LOQ

Detection limit and limit of quantification were found to be 50 and 200 ng spot⁻¹ respectively, which indicate adequate sensitivity of the method. The LOD and LOQ values for the proposed method were by one magnitude order lower than those reported by Kamdar et al.[12]; the latter might be due to the stronger pronounced effective diffusion of the lacosamide spot at higher Rf value 0.55 as against 0.24 ± 0.02 in our proposed method. The chromatograms of LOD and LOQ are shown in (Fig.6) and (Fig.7) respectively.

![Figure 6: Chromatogram of LOD for lacosamide](image)

![Figure 7: Chromatogram of LOQ for lacosamide](image)
CONCLUSION

The developed method is specific, accurate and robust for the determination of lacosamide content in the samples. Statistical data analysis proved the reproducibility and selectivity of the developed method for the analysis of lacosamide. The proposed HPTLC method has certain advantages over the reported HPTLC method such as high selectivity, sensitivity, low limit of detection and quantification. Moreover, the solvent consumption along with short analytical run time leads to a cost effective HPTLC method as per ICH guidelines as compared with HPLC method and seems to be suitable for routine analysis of pharmaceutical formulations in quality-control laboratories, where economy and speed are essential. Further, the proposed method can be extended to study the degradation of lacosamide under different stress conditions as per ICH guidelines.

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