STABILITY-INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR QUANTIFICATION OF NEW ANTI-EPILEPTIC DRUG LACOSAMIDE IN BULK AND PHARMACEUTICAL FORMULATION

An isocratic stability indicating reversed-phase liquid chromatographic determination was developed for the quantitative determination of lacosamide in the pharmaceutical dosage form. A Hypersil C-18, 4.5 μm column with mobile phase containing acetonitrile-water (20:80, v/v) was used. The flow rate was 1.0 mL min⁻¹ and effluents were monitored at 258 nm. The retention time of lacosamide was 8.9 min. The method was found to be linear in the concentration range of 5-100 μg/ml and the recovery was found to be in the range of 99.15-100.09%. The limit of detection and limit of quantification were found to be 2 and 5 μg/ml, respectively. Lacosamide stock solutions were subjected to acid and alkali hydrolysis, chemical oxidation and dry heat degradation. The drug was found to be stable to the dry heat and acidic condition attempted. The proposed method was validated and successfully applied to the estimation of lacosamide in tablet dosage forms.

Keywords: lacosamide; forced degradation; reversed phase liquid chromatography; validation.

Antiepileptic drugs are the mainstay of epilepsy management and, since 1989, 14 new antiepileptic drugs were approved for clinical use worldwide [1]. Lacosamide (LCM) is chemically \([\text{R}]-2\)-acetamido-\(N\)-benzyl-3-methoxypropionamide (Figure 1) and is a unique new antiepileptic drug with a molecular weight of 250.29 and an empiric formula of \(\text{C}_{13}\text{H}_{18}\text{N}_{2}\text{O}_{3}\). It was licensed in Europe and the United States in 2008 and 2009, respectively, for the adjunctive treatment of partial-onset seizures with or without secondary generalization in patients with epilepsy aged 16 years and older [2-4]. LCM is a synthetic chiral derivative of the amino acid D-serine [5] with a novel dual mode of action that is not shared with any other antiepileptic drug currently available [6]. It selectively enhances slow inactivation of voltage-gated sodium channels, resulting in stabilization of hyperexcitable neuronal membranes and inhibition of repetitive neuronal firing without exhibiting effects on fast activation that is expressed mainly in the nervous system and is thought to prevent axonal sprouting of neurones involved in seizure activity [6-8].

LCM is administered orally as film-coated tablets containing 50, 100, 150 or 200 mg in a syrup containing 15 mg/mL or in a 10 mg/mL solution for intravenous injection [9]. LCM has favorable pharmacokinetic characteristics, which allows ease of prescribing and the potential for good patient compliance. Pharmacokinetic parameters for LCM include: rapid and complete absorption from the gastrointestinal tract after oral ingestion (\(t_{\text{max}}\), 1-2 h, bioavailability = 100%); minimal protein binding (less than 30%); linear pharma-
cokineti cs; and moderate metabolism to pharmacologically inactive metabolites (40% excreted unchanged) with subsequent renal excretion and a half-life of 13 h [10-11]. The tablet formulations of LCM are bioequivalent to the intravenous injection formulation [12-13].

Therapeutic drug monitoring seeks to optimize patient outcome by managing their medication regimen with the assistance of information on the concentration of the antiepileptic drugs in blood (serum or plasma). Therapeutic drug monitoring attempts to optimize the desirable effects of an antiepileptic drug (seizure suppression) while keeping the undesirable adverse effects to a minimum [14].

A literature survey indicated only one method for the determination of LCM in human serum by HPLC [15]. Literature survey also reveals the saliva and serum concentration of lacosamide in patient with epilepsy [16]. This report describes the development and validation of a simple and robust high-performance liquid chromatographic (HPLC) method using UV detector for the quantification of LCM.

The International Conference on Harmonization (ICH) guidelines [17] require the implementation of stress testing procedures for the identification of degradation products that are potentially occurring in drug substances which can help to understand the possible degradation pathway for the drugs. No stability indicating method was reported for the estimation LCM, so attempt was made to develop stability indicating LC method for the estimation of LCM in pharmaceutical dosage form. This study reports forced degradation study of lacosamide under a variety of conditions such as acid and alkali hydrolysis, oxidative stress hydrolysis and dry heat degradation.

EXPERIMENTAL

Apparatus

A Series 200 HPLC system (Perkin-Elmer, Shelton, CT) equipped with a UV detector, Series 200 quaternary gradient pump, Series 200 column oven, manual injector rheodyne valve (7725i) with 20 µL fixed loop, Total chrome navigator software (version 6.1.1.0.0:K20), and Hypersil C18 column (150 mm×4.6 mm id, 5 µm particle size) was used. The analyte was monitored at 258 nm. All the drugs and chemicals were weighed on a Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

Chemicals and reagents

Analytically pure LCM was obtained as gift samples from Sun Pharmaceuticals Limited, Gujarat, India. HPLC grade acetonitrile, methanol and water were obtained from E. Merck Ltd., Mumbai, India. Tablet formulation lacoset - 50 (Sun pharmaceutical India Ltd., India) containing a labeled amount of 50 mg of lacosamide was procured from local market.

Chromatographic conditions

The Hypersil C18 column (150 mm×4.6 mm id, 5 µm particle size) was used at ambient temperature. The mobile phase consisted of acetonitrile-water (20:80, v/v) and the flow rate was maintained at 1 mL/min. The mobile phase was passed through nylon 0.45 µm - 47 mm membrane filter and degassed before use. The elution was monitored with UV detector at 258 nm, and the injection volume was 20 µL.

To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

Preparation of standard stock solutions

Accurately weighed 25 mg of LCM transferred to a 25 mL volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard solution of 1000 µg/mL. This solution (1 mL) was further diluted to 10 mL with the mobile phase to obtain a working standard stock solution of 100 µg/mL for the RP-HPLC method.

Method validation

The method was validated for accuracy, precision, linearity, specificity, detection limit, quantitation limit and robustness.

Linearity and range

Appropriate aliquot of LCM working standard solutions were taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 5, 10, 20, 30, 50 and 100 µg mL⁻¹ of LCM. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for both the drugs.

Precision

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of LCM (5, 30 and 100 µg mL⁻¹), and the results are reported in terms of relative standard deviation. The instrumental pre-
cision studies were carried out by estimating response of 3 different concentrations of LCM (5, 30 and 100 µg mL⁻¹) six times and results are reported in terms of relative standard deviation.

**Accuracy**

The accuracy of the method was determined by calculating recoveries of LCM by method of standard additions. Known amount of LCM (0, 10, 20 and 30 µg mL⁻¹) was added to a prequantified sample solution, and the amount of LCM was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

**Solution stability**

Stability of sample solutions were studied at 25±2 °C for 24 h.

**Robustness**

Robustness of the method was studied by deliberately changing the experimental conditions like flow rate and percentage of organic phase.

**Specificity**

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a preweighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Specificity was also studied by performing forced degradation study using acid and alkali hydrolysis, chemical oxidation and dry heat degradation studies and interference of the degradation products were investigated. LCM was weighed (10 mg each) and transferred to 10 mL volumetric flasks, dissolved in few ml of methanol and diluted up to the mark with methanol. These stock solutions were used for forced degradation studies.

a) **Alkali hydrolysis.** To the 10 mL volumetric flask, 1 mL stock solution of LCM was taken and 2.5 mL of 0.1 N NaOH was added to perform base hydrolysis. The flask was heated at 80 °C for 1 h and allowed to cool to room temperature. The solution was neutralized with 0.1 N HCl and diluted up to the mark with mobile phase. The appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 100 µg mL⁻¹ of LCM.

b) **Acid hydrolysis.** To the 10 mL volumetric flask, 1 mL stock solution of LCM was taken and 2.5 mL of 0.1 N HCl was added to perform acid hydrolysis. The flask was heated at 80 °C for 1 h and allowed to cool to room temperature. The solution was neutralized with 0.1 N NaOH and diluted up to the mark with mobile phase. The appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 100 µg mL⁻¹ of LCM.

c) **Oxidative stress degradation.** To perform oxidative stress degradation, the appropriate aliquot of stock solution of LCM was taken in 10 mL volumetric flasks and 2.5 mL of 3% hydrogen peroxide was added. The mixture was heated in a water bath at 80 °C for 1 h and allowed to cool to room temperature and diluted up to the mark with mobile phase. The appropriate aliquot was taken from above solution and diluted with mobile phase to obtain final concentration of 100 µg mL⁻¹ of LCM.

d) **Dry heat degradation.** Analytically pure sample of LCM was exposed in oven at 80 °C for 1 h. The solids were allowed to cool and 10 mg of LCM was weighed, transferred to volumetric flasks (10 mL) and dissolved in few ml of methanol. Volume was made up to the mark with the methanol. The solution was further diluted by mobile phase taking appropriate aliquots in 10 mL volumetric flask to obtain final concentration of 100 µg mL⁻¹ of LCM.

All the reaction solutions were injected in the liquid chromatographic system and chromatograms were recorded.

**Analysis of marketed formulation**

Twenty tablets were weighed and finely powdered. A mass equivalent to 25 mg of LCM was weighed and transferred in a 25 mL volumetric flask, mixed with methanol (10 ml), and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41, and the residue was washed thoroughly with methanol. The filtrate and washings were combined in a 25 ml volumetric flask and diluted to the mark with methanol. An aliquot of this solution (0.2 ml) was further diluted to 10 mL with methanol to obtain a solution containing 20 µg/mL of LCM and subjected to RP-HPLC analysis.

**Order of reaction of base hydrolysis of LCM**

In six 10 mL volumetric flasks, 1 mL stock solution of LCM was taken and 2.5 mL of 0.1 N NaOH was added to perform base hydrolysis. All the flasks were kept for base hydrolysis at 80 °C. At different time intervals (0, 10, 20, 30, 40 and 50 min) one volumetric flask was taken out and it allowed to cool in ice cold water to arrest the reaction. The solution was neutralized with 0.1 N HCl and diluted up to the mark with mobile phase. The appropriate aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 100 µg mL⁻¹ of LCM. All the reaction solutions were injected in the liquid chromatographic system and chromatograms were
recorded and peak area corresponding to drug peak was determined. The peak area of drug obtained at different time intervals was kept in 1st and 2nd order equation and rate constant were determined.

RESULTS AND DISCUSSION
Optimization of mobile phase
The objective of the method development was to resolve chromatographic peaks for active drug ingredients and degradation products produced under stressed conditions with less asymmetric factor.

Various mixtures containing aqueous buffer, methanol, and acetonitrile were tried as mobile phases in the initial stage of method development. Mixture of 0.02 M KH\textsubscript{2}PO\textsubscript{4}-methanol (45:55, v/v), methanol-water (80:20, v/v), methanol-water (60:40, v/v), methanol-acetonitrile-water (40:10:50, v/v/v), acetonitrile-water (30:70, v/v), acetonitrile-water (50:50, v/v) were tried as mobile phase but satisfactory resolution of drug and degradation peaks were not achieved.

The mobile phase acetonitrile-water (20:80, v/v) was found to be satisfactory and gave symmetric peak for LCM. The retention time for LCM was found to be 8.9 min as shown in Figure 2(A). The asymmetric factor and theoretical plates for LCM were found to be 0.91 and 5413, respectively. The mobile phase flow rate was maintained at 1 mL min\textsuperscript{-1}. The UV spectra of the drug showed that LCM absorbed appreciably at 258 nm, so detection was carried out at 258 nm.

Method validation
Linearity
The calibration curve for LCM was found to be linear in the range of 5-100 µg mL\textsuperscript{-1} with a correlation coefficient of 0.9999. The standard deviation value of slope of LCM was 2.44 which indicated strong correlation between peak area and concentration. The standard deviation value of intercept of LCM was 63.99.

Precision
Instrument precision was determined by performing injection repeatability test and the RSD value for LCM was found to be 0.96 %. The intra-day and inter-day precision studies were carried out and the % RSD were 0.17-1.32 and 0.42-1.28, respectively. The low RSD values indicate that the method is precise.

Accuracy
The accuracy of the method was determined by calculating recoveries of LCM by method of standard addition. The recoveries were found to be 99.15-100.09% for LCM (Table 1). The high values indicate that the method is accurate.

Limit of detection and limit of quantification
The detection limit and quantitation limit for LCM was 2.0±0.018 and 5.0±0.023 µg mL\textsuperscript{-1}, respectively. The above data shows that a microgram quantity of the drug can be accurately and precisely determined.

Specificity
The specificity study was carried out to check the interference from the excipients used in the formulation by preparing synthetic mixture containing the drug and excipients. The chromatogram showed peaks for the drug without any interfering peak. The % recovery was found to be 98.22-100.42%.

Forced degradation study
Chromatogram of base hydrolysis performed at 80 °C for 1 h showed complete degradation of LCM with degradation product peak at retention time (RT) 4.86 min and 10.52 (Figure 2B).

The chromatogram of acid hydrolysis performed at 80 °C for 1 h showed LCM was found to be stable.

Table 1. Accuracy study of the proposed method

<table>
<thead>
<tr>
<th>Amount of sample, µg/ml</th>
<th>Set</th>
<th>Amount drug of spiked µg/ml</th>
<th>Area (n = 3)</th>
<th>Average amount recovered, µg/ml</th>
<th>Recovery %</th>
<th>Mean recovery %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1</td>
<td>0</td>
<td>17895.78</td>
<td>20.02</td>
<td>99.08</td>
<td>100.09</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>18107.54</td>
<td></td>
<td>100.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>18237.14</td>
<td></td>
<td>100.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>10</td>
<td>27000.84</td>
<td>29.89</td>
<td>99.35</td>
<td>99.42</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>26830.62</td>
<td></td>
<td>98.40</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>27215.69</td>
<td></td>
<td>100.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>20</td>
<td>35971.66</td>
<td>39.83</td>
<td>98.86</td>
<td>99.15</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>35997.97</td>
<td></td>
<td>99.00</td>
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<td>3</td>
<td>20</td>
<td>36102.05</td>
<td></td>
<td>99.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>30</td>
<td>45243.65</td>
<td>49.86</td>
<td>100.04</td>
<td>99.28</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
<td>44956.33</td>
<td></td>
<td>98.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30</td>
<td>45118.16</td>
<td></td>
<td>99.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2. A) Liquid chromatogram showing chromatogram of lacosamide (LCM); B) chromatogram of base (0.1 N NaOH) treated lacosamide (LCM) at 80 °C for 1 h; C) Chromatogram of 3% hydrogen peroxide treated lacosamide (LCM) at 80 °C for 1 h.
The chromatogram of hydrogen peroxide at 80 °C for 1 h showed complete degradation of LCM with degradation product peak at retention time (RT) 10.75 min and 14.79 (Figure 2C). The chromatogram of dry heat degradation study at 80 °C for 2 h showed LCM was found to be stable.

The degradation study thereby indicated that LCM was found to be stable to acid hydrolysis, dry heat degradation study while it was susceptible to base hydrolysis and oxidative stress degradation (Table 2). No degradation products from different stress conditions affected determination of LCM.

**Solution stability**

The solution stability study showed that LCM was evaluated at room temperature for 24 h. The relative standard deviation was found below 2.0%. It showed that solution were stable up to 24 hrs at room temperature.

**Robustness**

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in Table 3. The low value of relative standard deviation was indicating that the method was robust.

### Order of reaction of base hydrolysis of LCM

From the forced degradation study it was revealed that LCM was susceptible to base hydrolysis. So, an experiment was carried out to determine order of reaction of base hydrolysis of drug. LCM was reacted with 0.1 M NaOH at 80 °C and samples were withdrawn at different time intervals and analyzed using the developed method. The peak area of drug obtained at different time intervals was kept in 1st and 2nd order equation and rate constant were determined. The value of rate constant at different time intervals for first order rate of reaction is shown in Table 4. The constant value of rate constant at different time intervals revealed, that LCM undergo base hydrolysis by first order rate of reaction:

$$k = \frac{2.303 \log \frac{a}{a-x}}{t}$$

where $k$ is the rate constant, $t$ is time in min, $a$ is the initial concentration of LCM, and $a-x$ is the concentration of LCM after time $t$.

### Analysis of marketed formulations

The proposed method was successfully applied to the determination of LCM in their tablet dosage form (Lacoset-50, Sun Pharmaceutical Ind. Ltd.). The % recovery for LCM was found to be 100.09±172.32 that is the mean value ± standard deviation of three samples.

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**Table 2. Forced degradation study of LCM for the proposed LC method (solutions were heated at 80 °C for specified period of time)**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time, h</th>
<th>Recovery, %</th>
<th>Retention time of degradation products, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base, 0.1 M NaOH</td>
<td>1</td>
<td>20.53</td>
<td>4.86, 10.52</td>
</tr>
<tr>
<td>Acid, 0.1 M HCl</td>
<td>1</td>
<td>99.34</td>
<td>-</td>
</tr>
<tr>
<td>3% Hydrogen peroxide</td>
<td>1</td>
<td>11.55</td>
<td>10.75, 14.79</td>
</tr>
<tr>
<td>Dry heat</td>
<td>2</td>
<td>100.54</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3. Data derived from robustness of lacosamide for the proposed LC method**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal condition</th>
<th>Change in condition</th>
<th>Change in RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate, mL/min</td>
<td>1.0 mL/min</td>
<td>0.9</td>
<td>0.97</td>
</tr>
<tr>
<td>Mobile phase ratio (acetonitrile:water, v/v)</td>
<td>20:80</td>
<td>25.75</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15:85</td>
<td>1.13</td>
</tr>
</tbody>
</table>

**Table 4. Rate constant of base hydrolysis of LCM**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Rate constant, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0.0203</td>
</tr>
<tr>
<td>20</td>
<td>0.0234</td>
</tr>
<tr>
<td>30</td>
<td>0.0191</td>
</tr>
<tr>
<td>40</td>
<td>0.0187</td>
</tr>
<tr>
<td>50</td>
<td>0.0224</td>
</tr>
</tbody>
</table>
determinations of 20 μg/mL of LCM and amount was recovered 20.02 μg/mL of LCM in the tablet dosage form which were comparable with the corresponding labeled amounts.

CONCLUSION

The proposed study describes stability indicating LC method for the estimation of LCM in bulk and their pharmaceutical dosage form. The advantage of the developed method is that it uses a simple mobile phase (acetonitrile:water) without buffer for the estimation of drug compared to reported method [15]. The reported method uses a complex drug extraction procedure from plasma while the developed method estimate LCM from tablet dosage form using a simple extraction procedure using methanol as solvent. The method was validated and found to be simple, sensitive, accurate and precise. Statistical analysis proved that the method was repeatable and selective for the analysis of LCM without any interference from the excipients. The method was successfully used for determination of drug in their pharmaceutical formulation. Additionally, the above results indicate the suitability of the method for acid, base, dry heat and wet heat degradation studies. As the method separates the drugs from its degradation products, it can be used for analysis of stability samples. The method is suitable for the routine analysis of LCM in tablets. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

Acknowledgements

The authors are thankful to Sun Pharmaceuticals Ltd., Baroda for providing gift sample of lacosamide. The authors are very thankful to SICART and Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Anand, for providing the necessary facilities to carry out this research work.

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METAĐA TEČNE HROMATOGRAFIJE ZA PRAČENJE STABILNOSTI I KVANTITATIVNU ANALIZU NOVOG EPILEPTIČNOG LEKA LACOSAMIDA


Razvijena je izokratska RP-HPLC metoda za praćenje njegove stabilnosti i kvantitativnu analizu lakcosamida u farmaceutskim oblicima. Korišćena je Hypersil C-18, 4.5 μm kolona. Mobilna faza je smeša acetonitrila i vode u odnosu 20:80 v/v. Brzina protoka mobilne faze je 1,0 ml min⁻¹, a komponente su detektovane na 258 nm. Retenciono vreme lakcosamida je 8,9 min. Nađeno je da metoda ima zadovoljavajuću linearnost u opsegu koncentracija 5-100 μg/ml sa odgovarajućim recovery vrednostima u opsegu 99.15-100.09%. Određeni su limit detekcije 2 μg/ml i limit kvantifikacije 5 μg/ml. Osnovni rastvor lakcosamida je podvrgnut kiseloj i alkalnoj hidrolizi, hemijskoj oksidaciji i degradaciji pri suvom zagrevanju. Nađeno je da zagrevanje i promena kiselosti ne utiče na stabilnost leka. Predložena metoda je validirana i uspešno primenjena za određivanje lakcosamida u obliku tableta.

Ključne reči: lakcosamid; forsirana degradacija; RP-HPLC; validacija.