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# RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LACOSAMIDE IN CONTROL RELEASE FORMULATIONS

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## ABSTRACT

This work has been done with a motto to develop a simple, accurate, precise, reproducible and economic reverse phase HPLC method for Lacosamide in bulk drug as well as in formulations. In the current developed method C8H column (150×4.6mm) was used as stationary phase and Sodium dihydrogen phosphate monohydrate buffer (pH 2.5) & Acetonitrile in a gradient mode as mobile phase. ICH guidelines were followed for method validation and different parameters studied include linearity range, system suitability, specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), Robustness and Solution Stability. The developed validated method was also utilized to quantify the Lacosamide in the marketed formulation successfully. The method was found linear within the range of 40-140µg/mL whereas recovery was found within the range of 99.50% -100.70%. Method was found to be able to distinguish the parent drug from degraded products & quantify Lacosamide in Dosage form (103.80%). A linear, robust & economic RP-HPLC method was developed within the good range which can be adopted for the routine analysis of Lacosamide in bulk drugs & formulations.

**Keywords: Lacosamide, Assay, HPLC, Validation, Accuracy**

## INTRODUCTION:

Over the last 25 years, the pharmacological management of the epilepsies has made tremendous progress, resulting in an increasing proportion of patients achieving seizure freedom without undue adverse effects [1]. A number of milestones have contributed to these advances. First of all, recognition of the value of monotherapy has been seminal in exploiting the therapeutic properties of individual antiepileptic drugs (AEDs) and in avoiding the acute and chronic toxicity associated with unnecessary polytherapy [2-3]. Several factors have influenced the selection of appropriate anti-epileptic drugs (AEDs) to treat epilepsy during the last decade. The side effect profiles of older AEDs are less acceptable when compared with newer AEDs [4]. Randomised controlled trials have directly compared the efficiency and tolerability of AEDs. For example, the SANAD trial recommended lamotrigine as first line treatment for focal epilepsies and sodium valproate as the first line treatment for generalised or unclassified epilepsy [5]. Several new effective AEDs have become available e.g. eslicarbazepine acetate, lacosamide, levetiracetam, oxcarbazepine, pregabalin, rufinamide, and zonisamide. Potentially harmful effects of certain AEDs on the cognitive and physical health of the unborn and developing child are increasingly recognised – in particular sodium valproate [5-6].

Lacosamide (LCM), (SPM 927, (R)-2-acetamido-N-benzyl-3-methoxypropionamide, previously referred to as harkoseride or ADD 234037) is a member of a series of functionalized amino acids that were specifically synthesized as anticonvulsive drug candidates. LCM has demonstrated antiepileptic effectiveness in different rodent seizure models and antinociceptive potential in experimental animal models that reflect distinct types and symptoms of neuropathic as well as chronic inflammatory pain. Rapidly and completely absorbed after oral administration, lacosamide has an elimination half-life of approximately 13 hours and a low potential for drug interactions. Additionally, lacosamide exhibits linear, dose-proportional pharmacokinetics with low intra- and interpatient variability. Randomized controlled trials of adjunctive lacosamide have demonstrated statistically significant reduction in median seizure frequency compared with placebo. In addition, 50%

responder rates for lacosamide were statistically superior to placebo. The most frequently reported adverse events included dizziness, headache, and nausea. A double-blind, double-dummy randomized trial of intravenous lacosamide as replacement for oral lacosamide showed that the safety and tolerability profiles were comparable for intravenous and oral lacosamide. The efficacy and safety results from completed clinical trials, as well as the favorable pharmacokinetic profile, suggest that lacosamide may represent a significant advance in antiepileptic drug therapy [7-8].

Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [9–12].

Literature survey revealed a few methods reported for determination of lacosamide in combination with other drugs in bulk drug as well as pharmaceutical preparation [13–16].

In this research, a new sensitive and rapid HPLC method was developed for the determination of lacosamide in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

## MATERIALS AND METHODS:

### Chemicals

HPLC-grade solvents such as acetonitrile, Sodium dihydrogen phosphate monohydrate and orthophosphoric acid and water were obtained from Merck Ltd. Bangalore India. Standards were purchased from Natural Remedies Ltd. Bangalore India [**Table 1**].

**Table 1: Standard and Test used for validation studies**

| Sr. No | Name of Standard/Test | Batch No | Potency (%) |
|---|---|---|---|
| 1 | Lacosamide WS | Sigma-Aldrich | 99.67 |
| 2 | Lacosamide 50 mg | IP-032/0356 | NA |
| 3 | Tablet Placebo | NA | NA |

### Preparation of standard solution

Transfer 50 mg of lacosamide working standard / reference standard into a volumetric flask and weigh precisely. Add 25 mL of diluent to the mixture. For around 5 minutes, sonicate the standard to verify that it is thoroughly dissolved. Mix well after diluting to volume with diluent Diluted 10 mL of stock solution in a 50-mL volumetric flask with diluent and mix.

**Preparation of test solution**

In a 100-mL volumetric flask, place 10 tablets and 50 mL diluent. Shake vigorously at 200 rpm for 60 minutes. To thoroughly dissolve the pills, sonicate for a further 15 minutes with intermittent shaking. Allow to come to room temperature before adding diluent and thoroughly mixing. Allow for the settling of any remaining solids. A 0.45 µ Prefilter + PTFE Syringe filter should be used to remove the supernatant solution. The first 2-3 mL of filtrate should be discarded, and the remaining filtrate should be discarded. Obtained 4 mL of clear filtrate, dilute to volume, then combine in a 100 mL volumetric flask with diluent.

**Chromatographic conditions for HPLC**

HPLC was performed using a Waters 2695 Alliance system with a 2996 photodiode array detector (PDA) and 2489 UV/Visible detector (UV). The standards as lacosamide were resolved on a reverse-phase column Inert Sustain C8, 150 X 4.6 mm, 3 µ (Mumbai, India). The mobile phase was prepared from 50 milimole of Sodium dihydrogen phosphate monohydrate in water and adjusted the pH 2.5 with diluted OPA (solvent-A) and Acetonitrile (100%) (solvent-B). The gradient program used is given in **Table 2**. The mobile phase flow rate was kept at 1 ml/min. Before the first injection, the column was saturated for 30 min with the initial mobile phase. Temperature was maintained at 25°C. Injection volume was decided to maintain at 20 µL. The PDA was set by optimizing wavelength to give best response for two peaks at 210 nm to acquire the chromatogram. The standard lacosamide were identified by comparing the retention time and spectra obtained from the sample and standard solutions. The present work was performed in an air-conditioned room maintained at 25°C [17-19].

**Table 2: Details of Gradient programme**

| Time (minute) | Flow (mL/minute) | % solvent A | % solvent B |
|---|---|---|---|
| 0 | 1.0 | 80 | 20 |
| 25 | 1.0 | 20 | 80 |
| 30 | 1.0 | 50 | 50 |
| 40 | 1.0 | 50 | 50 |
| 45 | 1.0 | 80 | 20 |
| 50 | 1.0 | 80 | 20 |

### Preparation of Calibration Graph

The linearity of peak area response for lacosamide was determined from 50 % to 150 % level of working concentration for lacosamide. The stock solutions of lacosamide were diluted in seven different known concentrations. Graphs of concentration (as x-value) versus area (as y-value) were plotted.

## VALIDATION OF HPLC METHOD

The proposed HPLC method was validated in terms of specificity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), standard solution stability, sample solution stability and robustness as per the International Conference on Harmonization (ICH) guidelines [20-23].

### Specificity

The specificity of the method was studied by assessment of peak purity of lacosamide using the Waters empower software and diode array detector and represented in terms of purity angle, purity threshold, and purity flag.

### Precision

Precision was studied in terms of system precision, method precision, and intermediate precision.

### System precision

System precision was carried out by six replicate injections from the same vial of standard and was expressed in terms of percent relative standard deviation (% RSD) tailing, plate count, and resolution.

### Method precision

The sample was analyzed for six times by mentioned procedure. The % assay for each analyte was expressed in terms of % RSD.

### Intermediate precision

Intermediate precision was performed on different systems, one the Waters e2695 Alliance system with a 2996 PDA and the other a 2489 ultraviolet (UV) detector by different analysts by analyzing six different sample of extract and was expressed in terms of % RSD.

### Recovery studies

The accuracy of the method was determined from recovery studies by adding a known amount of each standard at the 80%, 100%, and 120% level to the pre-analyzed sample followed by replicate quantitative analyses by the proposed method.

### Robustness

Robustness of the method was determined by slight deviation in the method parameters. The parameters selected were deviation in column chemistry, wavelength, column temperature, flow rate, and mobile phase gradient. The retention time of lacosamide, was determined and % RSD using system suitability parameters was observed.

Polyherbal tablet formulation was analyzed to determine the contents of lacosamide as per method described under chromatographic conditions by HPLC. All analysis was repeated three times and results were expressed in mean ± SD.

## RESULTS AND DISCUSSION

The composition of the mobile phase in the HPLC method was optimized by testing different solvent compositions of varying polarity, column chemistry, column temperature, and pH of mobile phase, and the best results were obtained by using the present method, which produces highly symmetrical peaks showing good resolution between each standard and other peaks **[Figure 1]**. The scanning wavelength selected was 210 nm to provide comparable results and at this wavelength all analyte showed optimum response. lacosamide were satisfactorily resolved with retention time about 2.7 minutes.

The calibration graph was in 50 % to 150 % level of working lacosamide, with acceptable correlation coefficients 0.9990 (40-140 µg/ml) **[Table 3]**. The graph for each standard is given in **Figure 2**.

The values of system precision, method precision, and intermediate precision are given against sample application and scanning of peak area and are expressed in terms of % RSD. For system precision % RSD values were found to be 0.2 % for lacosamide. Method precision was done and % RSD value were found to be 0.7 % for lacosamide respectively. For intermediate precision % RSD values between the two analysts were found to be 0.4 % for lacosamide. For the values of system precision, method precision, and intermediate precision, the %RSD values showed that the proposed method provides an acceptable level of system precision, method precision, and intermediate precision.

The peak purity of for each analyte was assessed by comparing their respective spectra at peak start, peak apex, and peak end positions of the spot from standard and extracts. The purity angle and purity threshold values are given in table **[Table 4].**

The given method was optimized by doing robustness. The peak area for each analyte was calculated for each parameter and % RSD was found to be less than 2%. The values of % RSD as shown in **Table 5** indicate better robustness of the method.

The recovery study was carried out by spiking known amount of standards into placebo solution at 80%, 100% and 120% of working concentration. The overall recovery percent were found to be for Lacosamide 100.71%. **[Table 6]**

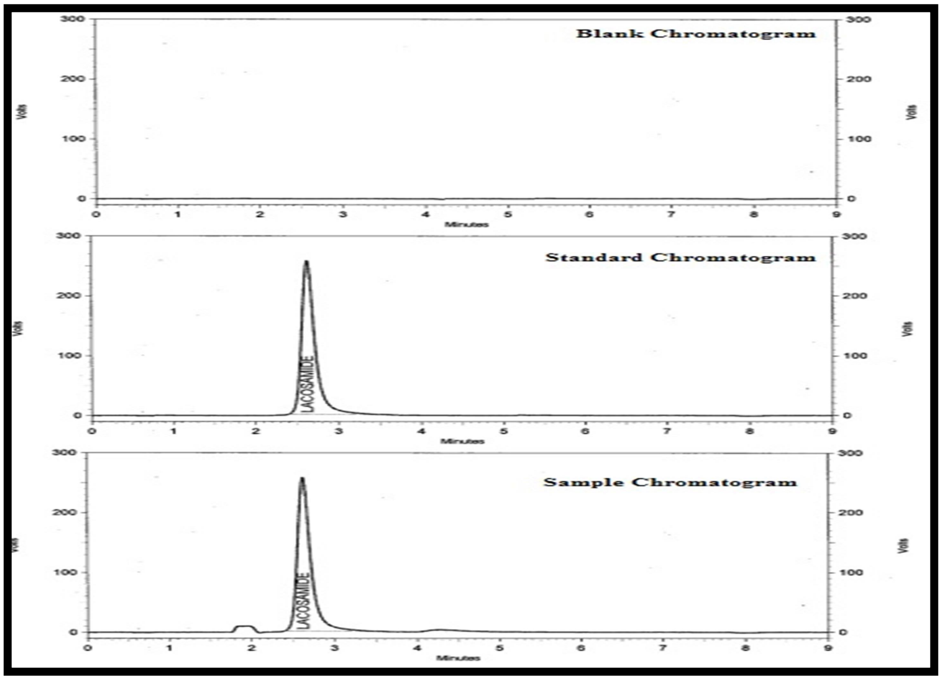

**Figure 1: Chromatograms for Blank, Standard and Sample**

**Table 3: Linearity of Lacosamide**

| % Level | Conc. of lacosamide (ppm) | Average Peak area of lacosamide |
|---|---|---|
| 50 | 44 | 759987 |
| 60 | 56 | 1098763 |
| 70 | 72 | 1563785 |
| 100 | 88 | 2073686 |
| 120 | 104 | 2599187 |
| 140 | 120 | 3098765 |
| 150 | 136 | 3578693 |
| $R^2$ | 0.9990 | |
| Slope of Regression line | 63183 | |
| y-intercept | 30961 | |

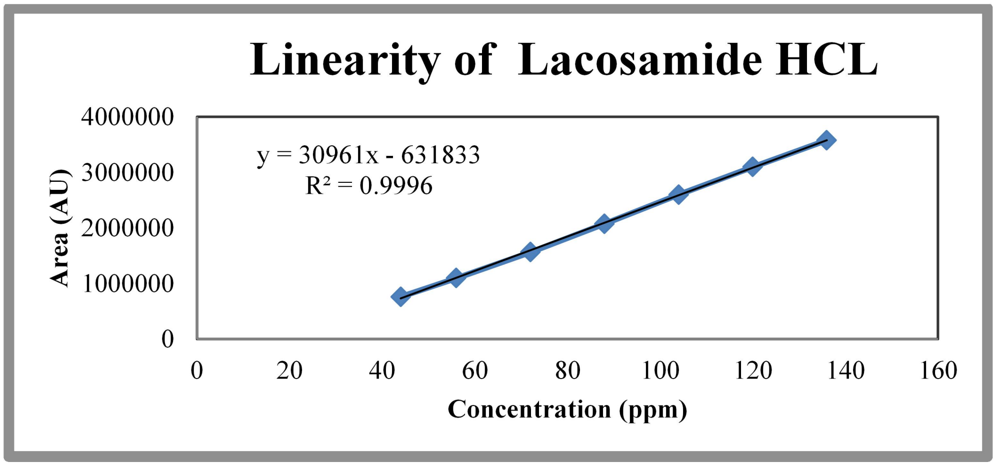

**Figure 2: Linearity graphs for standard**

**Table 4: Specificity of Lacosamide**

| Sample Name | Retention Time (Min) | Purity Angle | Purity Threshold | Peak Purity |
|---|---|---|---|---|
| **Blank (diluent)** | | | | |
| Lacosamide | ND | NA | NA | NA |
| **Standard solution** | | | | |
| Lacosamide | 2.724 | 0.116 | 0.280 | Pass |
| **Placebo solution** | | | | |
| **For Lacosamide Tablets 50 mg** | | | | |
| Lacosamide | ND | NA | NA | NA |
| **Worst Case Placebo** | | | | |
| Lacosamide | ND | NA | NA | NA |

**Table 5: Robustness for Lacosamide**

| Robustness parameter | | % RSD | Peak tailing | Theoretical plates | Remark |
|---|---|---|---|---|---|
| **Lacosamide** | | | | | |
| Wavelength (nm) | 205 | 0.36 | 1.34 | 32927 | Pass |
| | 210 | 0.26 | 1.30 | 31908 | Pass |
| | 215 | 0.58 | 1.32 | 32611 | Pass |
| Temperature (°C) | 25 | 0.33 | 1.31 | 31926 | Pass |
| | 30 | 0.60 | 1.39 | 35970 | Pass |
| | 35 | 0.26 | 1.30 | 32453 | Pass |
| Flow (mL/min) | 0.9 | 0.76 | 1.32 | 21839 | Pass |
| | 1.0 | 0.49 | 1.30 | 32869 | Pass |
| | 1.1 | 0.34 | 1.31 | 32205 | Pass |

**Table 6: Recovery for Lacosamide**

| Level | Concentration of Lacosamide w.r.t test concentration (%) | Amount of Lacosamide Added (mg) | Amount of Lacosamide Recovered (mg) | % Recovery | Mean % Recovery | % RSD | Overall % recovery and overall % RSD |
|---|---|---|---|---|---|---|---|
| 1 | 50 | 124.02 | 124.84 | 100.7 | 100.7 | 0.0 | Overall % recovery = 100.0<br>Overall % RSD = 0.6 |
| | | 124.11 | 125.03 | 100.7 | | | |
| | | 124.06 | 124.98 | 100.7 | | | |
| 2 | 100 | 248.17 | 248.05 | 99.9 | 99.8 | 0.1 | |
| | | 248.15 | 247.82 | 99.9 | | | |
| | | 248.32 | 247.51 | 99.7 | | | |
| 3 | 150 | 371.99 | 369.37 | 99.3 | 99.5 | 0.2 | |
| | | 372.06 | 371.09 | 99.7 | | | |
| | | 372.12 | 370.07 | 99.4 | | | |

## CONCLUSION:

The present investigation resulted in the development of an RP-HPLC-UV-DAD analysis method for Lacosamide that was validated in terms of linearity, precision, accuracy, specificity, system suitability and robustness. The presented method in addition to its novelty for determination of two ingredients i.e. Lacosamide at single wavelength is sufficiently rapid, simple and sensitive as well as precise and accurate that complies with ICH guidelines. The assay of the two active ingredients was not interfered by the excipients in the Tablet. Therefore, the proposed analytical method is recommended for the routine analysis of Lacosamide as such, or in various dosage forms. In addition, the method can be applied in many developing countries or field stations where advanced analytical equipment's are not available.

### Authors' contribution

Gopeshkumar Singh contribution included collecting samples, designing and performing laboratory work, analyzing the results, and preparing the paper. Gopeshkumar Singh and Vimal Kumar contribution included data interpretation and identification of the compounds. All the authors have read the final manuscript and approved the submission.

### Conflicts of interest

The authors declare no conflicts of interest.

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