



DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF LACOSAMIDE IN BULK AND ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-LC) method has been developed and subsequently validated for the determination of Lacosamide in Bulk and its pharmaceutical formulation. Separation was achieved with a Develosil ODS HG-5 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 μ m)) Column and Sodium di-hydrogen phosphate monohydrate buffer (pH adjusted to 3.0 with diluted orthophosphoric acid): Acetonitrile (700:300) v/v as eluent at flow rate 1.0 mL/min and the Column temperature was 40°C. UV detection was performed at 210nm and sample temperature was maintained at 5°C. The method is simple, rapid, and selective. The described method of Lacosamide is linear over a range of 3.996 μ g/mL to 47.952 μ g/mL. The method precision for the determination of assay was below 2.0%RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 100.0 to 101.3%. The method is useful in the quality control of Bulk and pharmaceutical formulations.

Key Words: LC Determination, Lacosamide.

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INTRODUCTION

Lacosamide tablets are indicated as adjunctive therapy in the treatment of partial-onset seizures in patients with epilepsy aged 17 years and older¹⁻². The chemical name of lacosamide, the single (R)-enantiomer, is (R)-2-acetamido-N-benzyl-3-methoxypropionamide (IUPAC). Lacosamide is a functionalized amino acid. Its molecular formula is C₁₃H₁₈N₂O₃ and its molecular weight is 250.30. Lacosamide is a white to light yellow powder. It is sparingly soluble in water and slightly soluble in acetonitrile and ethanol.

It is not official in any pharmacopoeia, few liquid chromatography procedures have been reported for the determination of Lacosamide³⁻⁴. The author have developed a liquid chromatographic method which would serve as a rapid and reliable method for the determination of Lacosamide in Bulk and pharmaceutical dosage forms.

EXPERIMENTAL⁵⁻⁷

Instrumentation

The analysis of the drug was carried out on a waters LC system equipped with 2695 pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Develosil ODS HG-5 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 μ m)) was used. The output of signal was monitored and integrated using waters Empower 2 software.

Chemicals and solvents

HPLC Grade water (Millipore), Acetonitrile (HPLC Grade), Orthophosphoric acid (HPLC Grade) and Sodium dihydrogen phosphate monohydrate of GR Grade were obtained from E. Merck (India) Ltd., Mumbai.

Buffer preparation

Accurately weigh and transfer about 1.37 grams of Sodium di-hydrogen phosphate monohydrate in 1000 mL of purified water and mix. Adjust pH to 3.0 (\pm 0.05) with dilute orthophosphoric acid solution. Filter the solution through 0.45 μ m membrane filter.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 700:300 v/v respectively.

Diluent preparation

Use Mobile phase as diluent.

Standard preparation: (For Lacosamide Tablets 200mg)

Accurately weigh and transfer about 40.0mg of Lacosamide working standard into a 100 mL volumetric flask, add 60 mL of diluent and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluent. Transfer 2.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.

Sample preparation: (For Lacosamide Tablets 200mg)

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 200 mg of Lacosamide into a 250 mL volumetric flask add about 100 mL of diluent, shake for 10minutes on orbital shaker and sonicate for 30minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter.

Transfer 2.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent.

Chromatographic conditions

A Develosil ODS HG-5 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 µm)) Column was used for analysis at column temperature 40°C. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10 µL and the sample temperature was maintained at 5°C. The photodiode array detector was set to a wavelength of 210nm for the detection and Chromatographic runtime was 10minutes.

Note: For Needle wash use Mobile phase.

RESULTS AND DISCUSSION

Method development⁵⁻⁷

To develop a suitable and robust LC method for the determination of Lacosamide, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Develosil ODS HG-5 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 µm)) with the following mobile phase. Accurately weigh and transfer about 1.37 grams of Sodium di-hydrogen phosphate monohydrate in 1000 mL of purified water and mix. Adjust pH to 3.0 (±0.05) with dilute orthophosphoric acid solution. Filter the solution through 0.45µm membrane filter. Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 600:400 v/v respectively. Lacosamide peak was eluted at void volume. For next trial the mobile phase composition was changed slightly.

The mobile phase composition was Buffer and Acetonitrile in the ratio of 800:200 v/v. Above trail the peak shape was broad. The mobile phase composition changed to respectively Buffer and Acetonitrile in the ratio of 700:300 v/v respectively as eluent at flow rate 1.0 mL/min and the Column temperature was 40°C. UV detection was performed at 210nm and the sample temperature was maintained at 5°C. The retention time of Lacosamide is 3.09 minutes (refer Fig-2.) and the peak shape was good.

The chromatogram of Lacosamide standard using the proposed method is shown in Fig-2. System suitability results of the method are presented in Table-1. Lacosamide shows significant UV absorbance at Wavelength 210nm. Hence this wavelength has been chosen for detection in analysis of Lacosamide.

Column selection

Based on the retention and better peak shape of the compound Develosil ODS HG-5 ((Make: Nomura chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 µm)) Column was selected as a suitable column for analysis of Lacosamide.

Method validation⁵⁻⁷

The developed LC method extensively validated for assay of Lacosamide using the following parameters.

Specificity

Blank and Placebo interference

A study to establish the interference of placebo was conducted. Assay was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of Blank and Placebo solutions showed no peaks at the retention time of Lacosamide peak. This indicates that the excipients used in the formulation do not interfere in estimation of Lacosamide in Lacosamide tablets.

The chromatogram of Lacosamide Blank and Placebo using the proposed method is shown in Fig- 3 & Fig-4.

Linearity of detector response

Linearity of detector response was established by plotting a graph to concentration *versus* average area and determining the correlation coefficient.

A series of solutions of Lacosamide standard were prepared in the concentration range of about 3.996 µg/mL to 47.952 µg/mL. A graph was plotted to concentration in µg/mL on X-axis *versus* response/Area on Y-axis. The detector response was found to be linear with a correlation coefficient of 1.0000. Linearity graph is shown in Fig-5. Linearity results of the method are presented in Table-2.

Precision of test Method

The precision of test method was conducted by assay in six samples of Lacosamide Tablets. The average % assay of Lacosamide in Lacosamide Tablets was found to be 100.0 for 200mg tablets and the %RSD is 0.5. The results were given in Table-3. A typical LC Chromatogram is shown in Fig-6.

Accuracy

A Study of recovery of Lacosamide from spiked placebo was conducted at five different spike levels i.e.50, 75, 100, 150 and 200%. Samples were prepared by mixing placebo with Lacosamide raw material equivalent to about the target initial concentration of Lacosamide. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table-4. The mean recoveries of Lacosamide from spiked were found to be in the range of 100.0-101.3%.

Ruggedness

A study to establish the stability of Lacosamide in standard and test solutions were conducted on bench top and refrigerator at Initial, 1 day and 2 day. The assay of Lacosamide in standard and test solutions were estimated against freshly prepared standard each time. The difference in% assay of standard and test solutions from initial to 1 day and 2 days was calculated and given in Table-5 & Table-6. From the above study, it was established that the Standard and sample preparations are stable for a period of 48hours at room temperature (25°C±2°C) and 48hours at refrigerator condition (2°C-8°C).

Robustness

A study to establish the effect of variation in mobile phase composition, flow, temperature and pH of Buffer in mobile phase was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system. The system suitability parameters and % assay were evaluated. From the above study the proposed method was found to be robust.

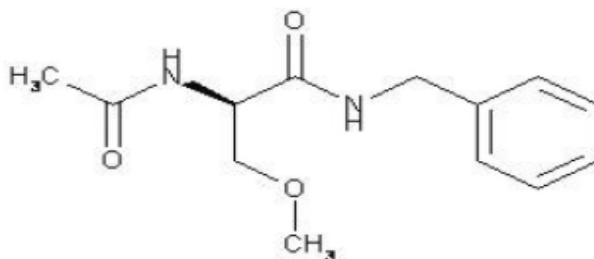


Fig.-1: Chemical Structure of Lacosamide

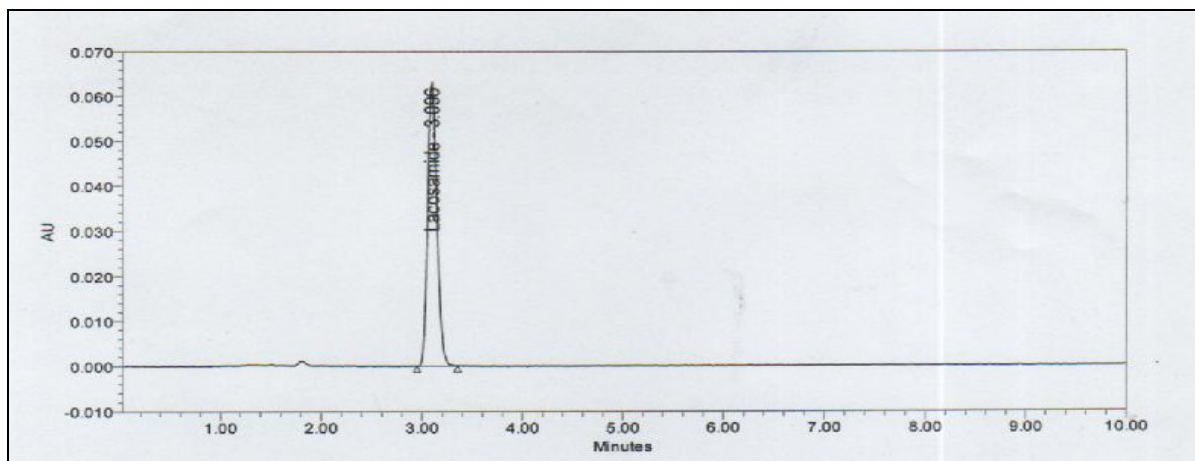


Fig-2: HPLC Chromatogram of Lacosamide Standard.

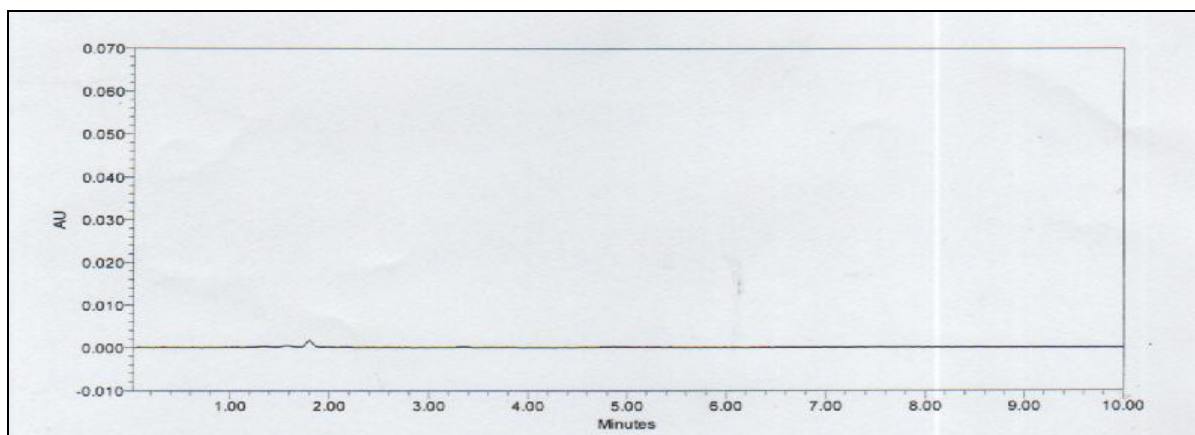


Fig-3: HPLC Chromatogram of Lacosamide Blank.

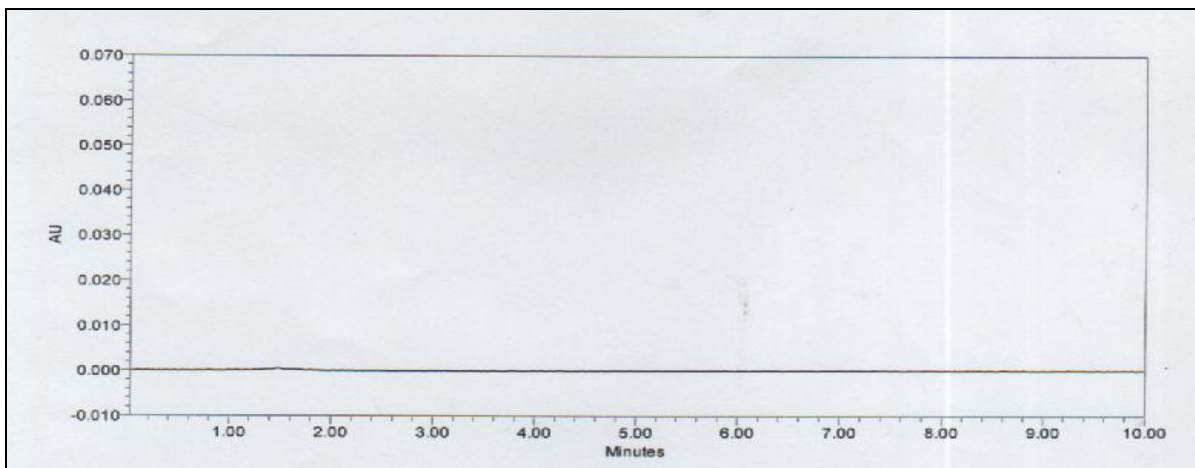


Fig-4: HPLC Chromatogram of Lacosamide placebo.

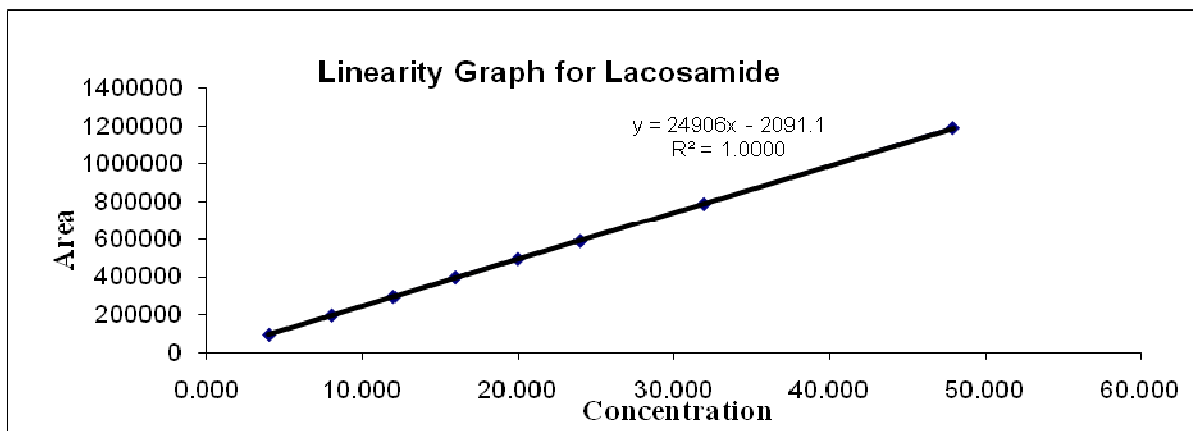


Fig-5: Linearity of detector response graph.

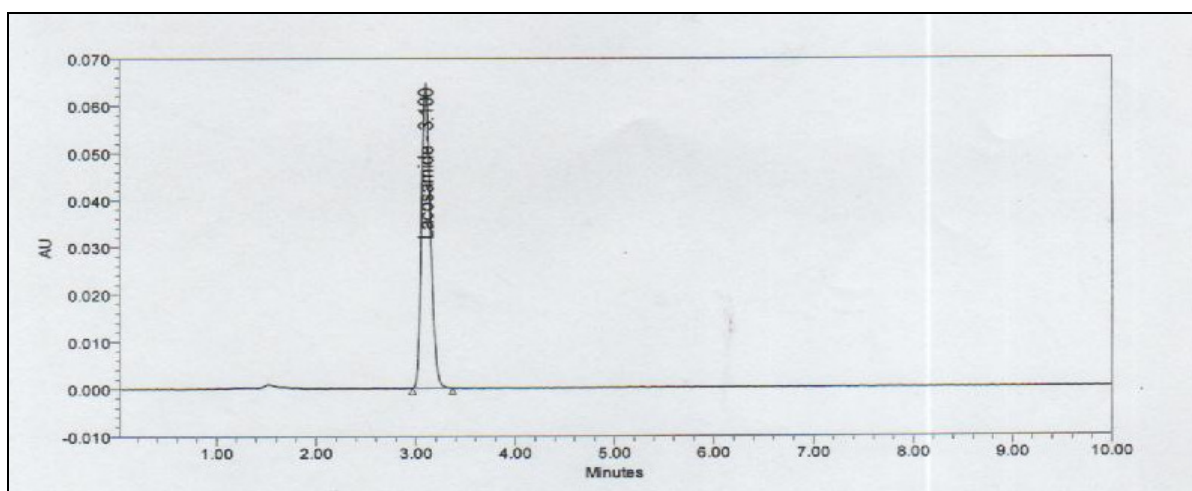


Fig-6: Typical LC chromatogram of Formulated Lacosamide 200mg Tablets.

Table-1: System suitability report

| Compound | Retention time*(min.) | Lacosamide area/response* | USP Tailing* | USP plate count* | %RSD* |
|------------|-----------------------|---------------------------|--------------|------------------|-------|
| Lacosamide | 3.096 | 397545 | 1.17 | 5533 | 0.13 |

*Number of standard injections analysed are six.

Table-2: Linearity Table Report

| S.No. | Concentration (mcg/mL) | Area | y-Best fit | (Difference) ² | Correlation Coefficient (R)= | 1.0000 |
|-------|------------------------|--------|------------|---------------------------|---|----------|
| 25% | 3.996 | 96024 | 97432 | 1982690 | Regression Coefficient (R ²)= | 1.0000 |
| 50% | 7.992 | 198695 | 196955 | 3026651 | y-Intercept= | -2091.1 |
| 75% | 11.988 | 296267 | 296478 | 44718 | Slope of Regression line= | 24906 |
| 100% | 15.984 | 398801 | 396002 | 7836314 | Residual Sum of squares= | 32081074 |
| 125% | 19.980 | 496396 | 495525 | 758901 | Minimum Con in mcg/mL = | 3.9960 |
| 150% | 23.976 | 591734 | 595048 | 10982885 | Maximum Con in mcg/mL = | 47.9520 |

| | | | | | | |
|------|--------|---------|---------|---------|------------------------------|--------|
| 200% | 31.968 | 791941 | 794094 | 4637256 | y-Intercept at 100 % level = | -0.524 |
| 300% | 47.952 | 1193864 | 1192187 | 2811659 | | |

Table-3: results for precision of test method

| Sample No. | % of Lacosamide in Lacosamide Tablets (200mg) |
|------------|---|
| 01 | 99.3 |
| 02 | 100.8 |
| 03 | 100.1 |
| 04 | 99.9 |
| 05 | 100.3 |
| 06 | 99.5 |
| Average | 100.0 |
| SD | 0.5456 |
| % RSD | 0.5 |

Table-4: Accuracy in the assay determination of Lacosamide

| Sample No. | Spike level | 'mcg/mL' added | 'mcg/mL' found (recovered) | % of Recovery | Mean % recovery |
|------------|-------------|----------------|----------------------------|---------------|-----------------|
| 1. | 50% | 7.9759 | 8.0087 | 100.3 | 100.6 |
| 2. | 50% | 7.9600 | 8.0064 | 100.5 | |
| 3. | 50% | 7.9122 | 8.0170 | 101.1 | |
| 4. | 75% | 11.9360 | 11.9416 | 99.7 | 100.2 |
| 5. | 75% | 11.7928 | 11.9520 | 101.8 | |
| 6. | 75% | 11.8167 | 11.6751 | 99.0 | |
| 7. | 100% | 15.9120 | 16.0746 | 101.2 | 101.3 |
| 8. | 100% | 15.8881 | 16.0726 | 101.3 | |
| 9. | 100% | 15.8642 | 16.0633 | 101.5 | |
| 10. | 150% | 23.8640 | 23.8511 | 100.2 | 100.0 |
| 11. | 150% | 23.8401 | 23.8438 | 99.8 | |
| 12. | 150% | 23.8162 | 23.8307 | 99.9 | |
| 13. | 200% | 31.8478 | 31.9206 | 100.2 | 100.2 |
| 14. | 200% | 31.8319 | 31.9176 | 100.2 | |
| 15. | 200% | 31.8557 | 31.9445 | 100.1 | |

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Lacosamide and can be reliably adopted for routine quality control analysis of Lacosamide in Bulk and its pharmaceutical formulations.

Table-5: Bench top Stability of Lacosamide Test preparation and Standard Preparation

| Time | % Assay of Standard preparation | Difference | % Assay of test preparation | | Difference | |
|----------------|---------------------------------|------------|-----------------------------|--------|------------|--------|
| | | | Test-1 | Test-2 | Test-1 | Test-2 |
| Initial | 99.9® | NA* | 100.0 | 100.8 | NA* | NA* |
| After 24 hours | 99.5 | 0.4 | 99.5 | 100.4 | 0.5 | 0.4 |
| After 48 hours | 98.9 | 1.0 | 99.0 | 99.7 | 1.0 | 1.1 |

NA*:Not Applicable

®: Potency of Lacosamide on as is basis.

Table-6: Refrigerator Stability of Lacosamide Test preparation and Standard Preparation

| Time | % Assay of Standard preparation | Difference | % Assay of test preparation | | Difference | |
|----------------|---------------------------------|------------|-----------------------------|--------|------------|--------|
| | | | Test-1 | Test-2 | Test-1 | Test-2 |
| Initial | 99.9® | NA* | 100.0 | 100.8 | NA* | NA* |
| After 24 hours | 99.0 | 0.9 | 99.2 | 100.4 | 0.8 | 0.4 |
| After 48 hours | 98.8 | 1.1 | 99.0 | 99.7 | 1.0 | 1.1 |

NA*:Not Applicable

®: Potency of Lacosamide on as is basis.

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