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Development and Validation of Stability Indicating RP-LC Method for Estimation of Lacosamide in Bulk and Its Pharmaceutical Formulations

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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 Xterra RP-8 ((Make: Waters Corporation; 150 mmx4.6 mm I.D; particle size 5 µm)) Column and Sodium di-hydrogen phosphate monohydrate buffer (pH adjusted to 3.0 with diluted orthophosphoric acid): Acetonitrile (800:200) v/v as eluent at a flow rate of 1.0 ml/min. UV detection was performed at 230nm. The method is simple, rapid, and selective. The described method of Lacosamide is linear over a range of 12.0µg/ml to 37.85 µg/ml. The method precision for the determination of assay was below 1.0%RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 99.3 to 100.9%. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of Lacosamide in bulk, its dosage forms.

Key Words: Lacosamide, RP-LC, Validation, Dosage form.

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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 older[1-2]. The chemical name of lacosamide, the single (R)-enantiomer, is (R)-2-acetamido-N-benzyl-3methoxypropionamide (IUPAC). Lacosamide is a functionalized amino acid. Its molecular formula is $C_{13}H_{18}N_2O_3$ 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 Lacosamide[3-4] .The authors 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.

The authors have developed a new, simple and fast analytical method by RP-LC to quantify Lacosamide in bulk and its dosage forms. This validation study is carried out as per ICH guidelines.

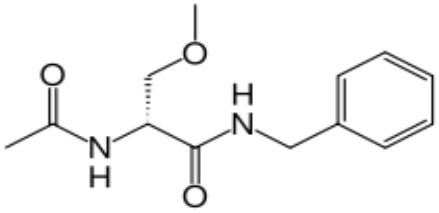


Figure-1: Chemical Structure of Lacosamide

MATERIAL AND METHODS[5-7]

Instrumentation

The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Xterra RP-8 ((Make: Waters Corporation, Ireland); 150 mmx4.6 mm I.D; particle size 5 µm) was used. The output of signal was monitored and integrated using waters Empower 2 software.

Chemicals and solvents

Milli-Q Water, Acetonitrile (HPLC Grade), Orthophosphoric acid (GR Grade), Sodium dihydrogen phosphate monohydrate (GR Grade) were obtained from Qualigens Ltd., Mumbai.

Buffer preparation

Accurately weigh and transfer about 2.72 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.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 800:200 v/v respectively.

Diluent preparation

Water and Acetonitrile in the ratio of 250:750 v/v is used as diluent.

Standard preparation:

Accurately weigh and transfer about 50.0mg of Lacosamide 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 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent.

Sample preparation: (For Lacosamide Tablets 500mg)

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent.

Chromatographic conditions

An Xterra RP-8 ((Make: Waters Corporation (Ireland); 150 mmx4.6 mm I.D; particle size 5µm)) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0ml/min. The sample injection volume was 100 µl. The photodiode array detector was set to a wavelength of 230nm for the detection and Chromatographic runtime was 10minutes.

RESULTS AND DISCUSSION

Method development[5-7]

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 Xterra RP-8 ((Make: Waters Corporation (Ireland); 150 mmx4.6 mm I.D; particle size 5 µm)) with the following mobile phase. Accurately weigh and transfer about 2.72 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 500:500 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 700:300 v/v. In the above trail also the retention time of the peak improved but not satisfactory.. Again the mobile phase composition changed slightly to Buffer and Acetonitrile in the ratio of 800:200 v/v respectively as eluent at flow rate 1.0 ml/min. UV detection was performed at 230nm. The retention time of Lacosamide was about 4.0 minutes (refer Figure-2.) and the peak shape was good.

The chromatogram of Lacosamide standard using the proposed method is shown in Figure-2. System suitability results of the method are presented in Table-1. Lacosamide shows significant UV absorbance at Wavelength 230nm. Hence this wavelength has been chosen for detection in analysis of Lacosamide.

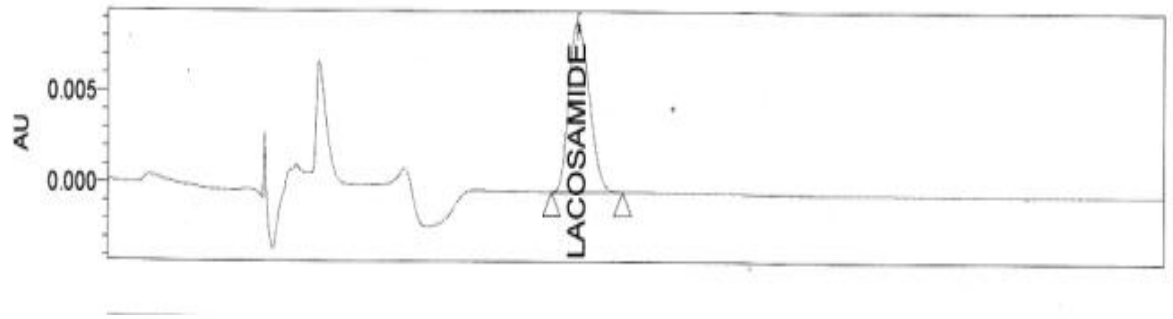


Figure 2: A typical HPLC Chromatogram showing the Peak of Lacosamide

Table 1: System suitability parameters for Lacosamide by proposed method

Name of the Compound	Theoretical plate	Tailing factor
Lacosamide	2405	1.26

Method validation [7-8]

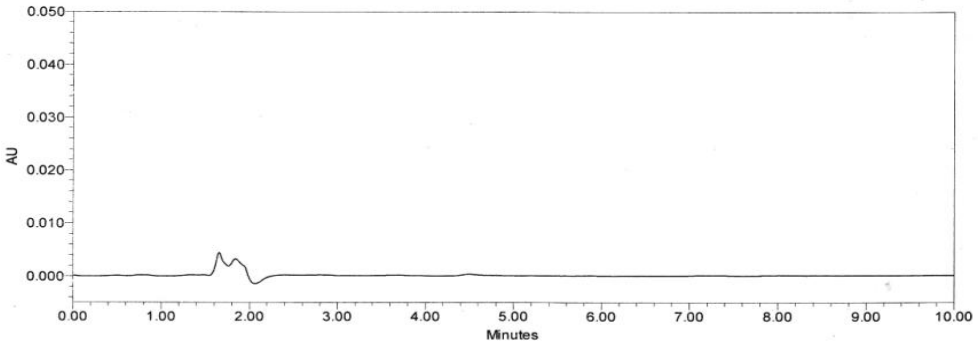
The developed RP-LC method extensively validated for the determination assay content of Lacosamide using the following Parameters.

Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in defined above chromatographic conditions and the blank chromatogram was recorded. Chromatogram of Blank solutions showed no peaks at the retention time of Lacosamide peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Lacosamide in Lacosamide tablets.

The chromatogram of Lacosamide Blank using the proposed method is shown in Figure 3.



	Peak Name	RT
1	LACOSAMIDE	4.400

Figure 3: A typical HPLC Chromatogram showing the no interference of diluent for Lacosamide

Forced Degradation:

Control Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent.(Figure 4)

Acid Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 10ml of 5N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 5N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 4)

Base Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 10ml of 5N Base (NaOH), refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 5N Acid (HCl) and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane

Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 4)

Peroxide Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 2ml of 30% Peroxide, refluxed for 30min at 60°C, then cooled to room temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 4)

Thermal Degradation Sample:

Powder collected from 20 tablets are exposed to heat at 105°C for about 5days. Then Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 50 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 4)

System and Method Precision

In the study of the instrumental system precision where, a RSD of 0.3% was obtained for the standard area obtained corresponding to the first day, being 0.6% for the second day, respectively. The method precision study for six sample preparations in marketed samples showed a RSD of 0.5% and the 95% confidence interval of 0.5 with the assay range of 98.2-99.4 with an average of 99.0

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 0.6 %(For Standard). The same study was carried out for different analysts (n = 6 number of samples per analyst) obtaining a RSD of 0.6 %(Intermediate Precision) and 95% confidence interval of 0.7 with the assay range of 98.6-100.3 with an average of 99.4. The Overall %RSD for n=12 is 0.6. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.(Table 2)

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Lacosamide and analyzed as per the proposed method.

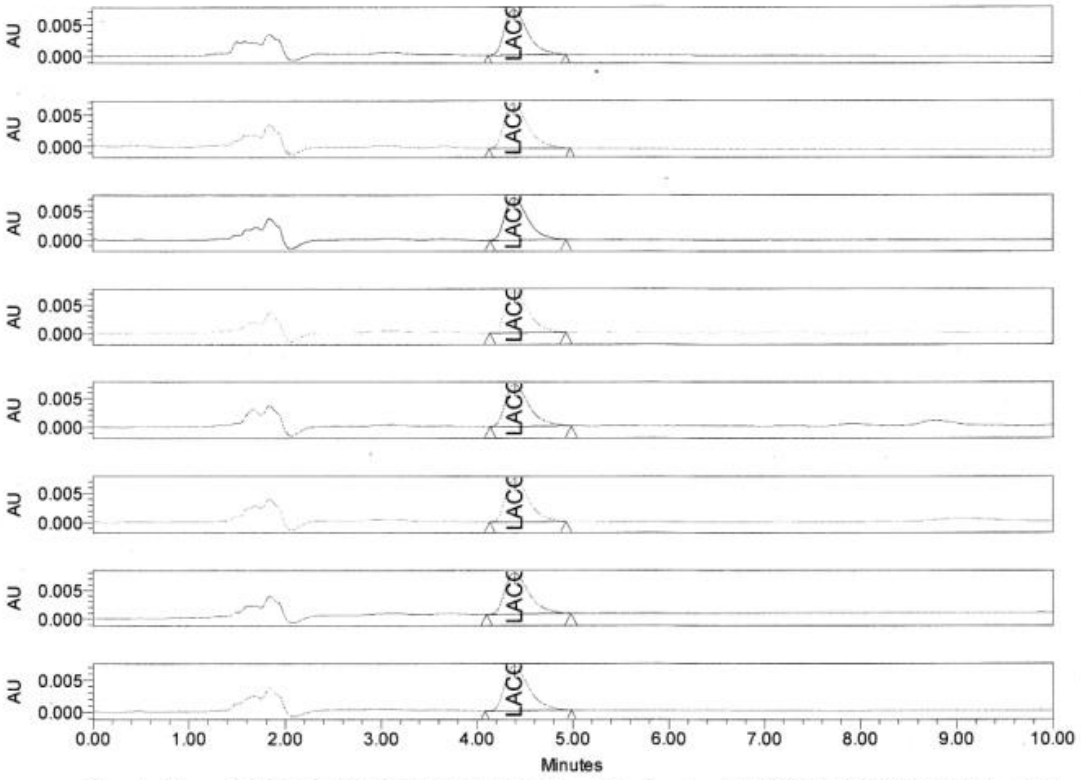


Figure 4: A typical HPLC Chromatogram showing the degradation profile of Lacosamide by proposed method.

Table 2: Method Precision (Inter and Intra) studies for Lacosamide by proposed method

Method Precision(Inter &Intra Day)	
99.1	99.60
99.3	100.3
98.6	99.5
99.1	99.6
99.4	98.8
98.2	98.6
Overall Average	99.2
Overage Std Dev	0.57
Over all %RSD	0.6

The percentage recoveries with found in the range of 99.3 to 100.9 with an overall %RSD of 0.60. From the data obtained which given in table-3 the method was found to be accurate.

Table 3: Recovery studies for Lacosamide by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	99.3-100.8	0.8	0.6
100	99.4-100.1	0.4	
150	99.7-100.9	0.6	

Table 4: Linearity studies for Lacosamide by proposed method

% Level (Approx.)	Concentration (µg/ml)	Average Abs.
50	11.95	70203
75	17.93	99781
100	23.9	126507
125	31.87	159996
150	37.85	189409
	Slope	4537
	Intercept	17121
	% Y-Intercept	10.7
	STYEX	1548
	CC	0.9996
	RSQ	0.9992
	Residual sum of squares	1548
	LLD	0.30
	LLQ	0.90

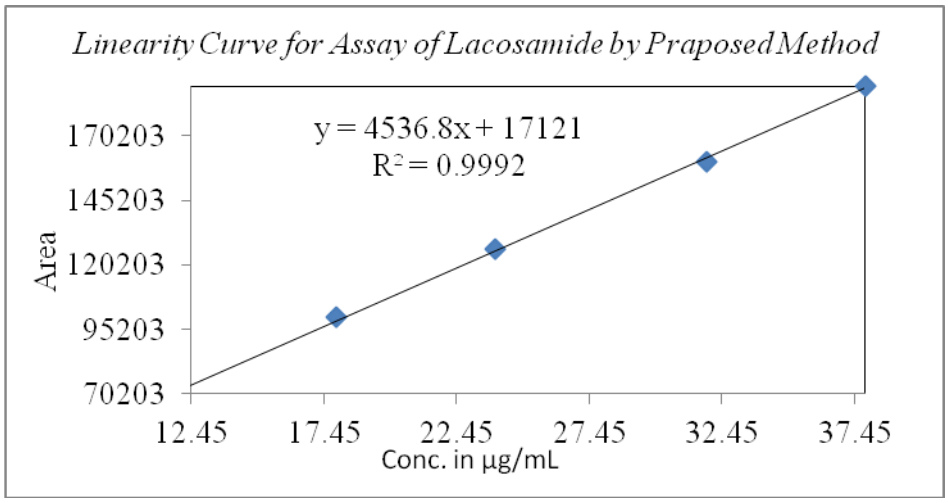


Figure 5: Calibration curve for Lacosamide

Linearity of detector response

The standard curve was obtained in the concentration range of 12-37.85µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation

coefficient [r^2] of standard curve were calculated and given in Table 4 and figure-5 to demonstrate the linearity of the method.

CONCLUSION

We have developed a fast, simple and reliable analytical method for determination of Lacosamide in pharmaceutical preparation using RP-LC. As there is no interference of blank at the retention time of Lacosamide. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of Lacosamide in bulk, its pharmaceutical dosage forms.

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