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Research Article

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Stability indicating RP-HPLC method for the estimation of lacosamide in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, selective, linear, precise, accurate and stability indicating RP-HPLC method is developed and validated for estimation of lacosamide in pharmaceutical dosage form. Isocratic elution at a flow rate of 1 mL/mL was employed on Kromasil C_{18} column at ambient temperature. The mobile phase consisted of acetonitrile and phosphate buffer (0.02M) in the ratio of 40:60 (v/v) and the effluent was monitored at 210 nm. Linearity was observed in concentration range of 5-50 µg/mL. The retention time was found to be 2.49 min. The method was validated as per the ICH guidelines. No chromatographic interference from tablet excipients was found. The proposed method was successfully applied for the estimation of lacosamide in pharmaceutical dosage forms.

Key words: Estimation, Method development, Lacosamide, RP-HPLC, Validation, Stability studies.

INTRODUCTION

Lacosamide (Figure 1) chemically described as N^2-acetyl-*N*-benzyl-D-homoserinamide used for the treatment of seizures [1]. It can also be prescribed off-label to treat conditions such as diabetic neuropathic pain. It selectively enhances slow inactivation of voltage-gated sodium channels, resulting in stabilization of hyper excitable neuronal membranes and inhibition of repetitive neuronal firing.

Literature survey reveals that few analytical methods [2-10] have been reported for the estimation of lacosamide. In the present study a simple, precise, accurate and stability indicating reversed-phase HPLC method was developed and validated for the estimation of lacosamide in pharmaceutical dosage form as per ICH/USP guideline validation norms [11, 12].

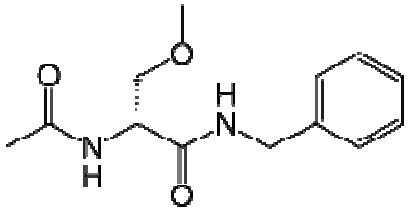

Figure 1: Chemical structure of lacosamide

EXPERIMENTAL SECTION

Instruments

The HPLC analysis was carried out on Waters HPLC system (2695 module**)** equipped with PDA detector (2996 module) with auto sampler and running on Waters Empower software. The column used is Kromasil C_{18} (150 × 4.6

mm, packed with 5 µm) and detection was performed at 210 nm. Ultra sonicator (Enertech), Electronic balance (Infra), pH analyzer (Elico), Filteration system (Reveria) was used to carry out the research work.

Reagents and chemicals

Lacosamide working standard was kindly gifted by Dr Reddy's Laboratories, Hyderabad. Tablets were purchased from local pharmacy manufactured by Sun pharmaceuticals (Lacoset). Acetonitrile (HPLC grade; Merck), water (HPLC grade; Milli-Q), sodium hydroxide (SD fine chemicals), hydrochloric acid (Finar chemicals), hydrogen peroxide (Alpha pharma), Potassium di hydrogen orthophosphate and ortho phosphoric acid (AR grade; SD fine chemicals) were used in the study.

Preparation of mobile phase

2.72 g of potassium dihydrogen orthophosphate was dissolved in 1000 mL of water, and the pH was adjusted to 2.5 using ortho phosphoric acid. This solution was sonicated to degas the buffer. 400 mL of acetonitrile and 600 mL of buffer were transferred in to a 1000 mL mobile phase bottle, mixed and sonicated up to 15 minutes to degas the mobile phase. Then it was filtered through 0.45 µm membrane filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard solution

10 mg of lacosamide reference standard was accurately weighed and transferred into a 10mL volumetric flask containing 7 mL of mobile phase. Then it was sonicated for 10 minutes to dissolve the drug completely. The volume was adjusted with the mobile phase to get stock solution of 1000 µg/mL. 0.2 mL of this stock solution was transferred into a 10 mL volumetric flask and the volume was made up to the mark with mobile phase. This solution was filtered through 0.45 µm membrane filter, which gives a solution of concentration 20 µg/mL.

Preparation of sample solution

Twenty tablets (Lacoset) were weighed and their average weight was calculated. A quantity of the tablet powder equivalent to 50 mg of lacosamide was transferred into a 50 mL volumetric flask containing 25mL of mobile phase and allowed to stand for 1hr with intermittent sonication to ensure complete solubility of the drug and then filtered through 0.45 µm membrane filter and the volume was adjusted up to the mark with mobile phase. Further 0.2 mL of the above stock solution was pipetteted into a 10 mL volumetric flask and the volume was adjusted up to the mark with mobile phase to give a concentration of 20µg/mL.

DEVELOPMENT OF NEW METHOD

A number of eluting systems were examined for optimization of the mobile phase. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of acetonitrile: phosphate buffer at the ratio of 40:60 (v/v). A flow rate of 1.0 mL/ min was found to be optimum which gives a retention time of 2.49 min with base line stability. The injection volume of sample was 20 µL and each of the dilution was injected six times and the run time was 6 minutes. From the chromatograms obtained, the retention times and area under the peaks of the drug were noted. The optimized chromatographic conditions are given in the table 1. A typical chromatogram of standard solutions of lacosamide shown in Figure 2.

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Table 1: Optimized chromatographic conditions of lacosamide

Parameters	Method
Stationary phase (column)	Kromasil C_{18} (150 × 4.6 mm, packed with 5 µm)
Mobile Phase	40:60 (Acetonitrile: Phosphate buffer)
Flow rate (mL/min)	1.0
Run time (minutes)	6.0
Column temperature ($^{\circ}$C)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	210
Drugs Rt (min)	2.495

METHOD VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity

From the standard stock solution, the various dilutions of lacosamide in the concentration of 5, 10, 15, 20, 25, 30, 40 and 50 µg/mL were prepared. Each of these solutions (20 µL) was injected six times in to the chromatographic system and the peak areas and retention times were recorded. Calibration curve was obtained by plotting the peak

area versus the applied concentrations of lacosamide. The linearity range and corresponding graph are shown in Table 2 and Figure 3. A good linear relationship (r^2=0.999) was observed between the concentration range of 5-50 µg/mL.

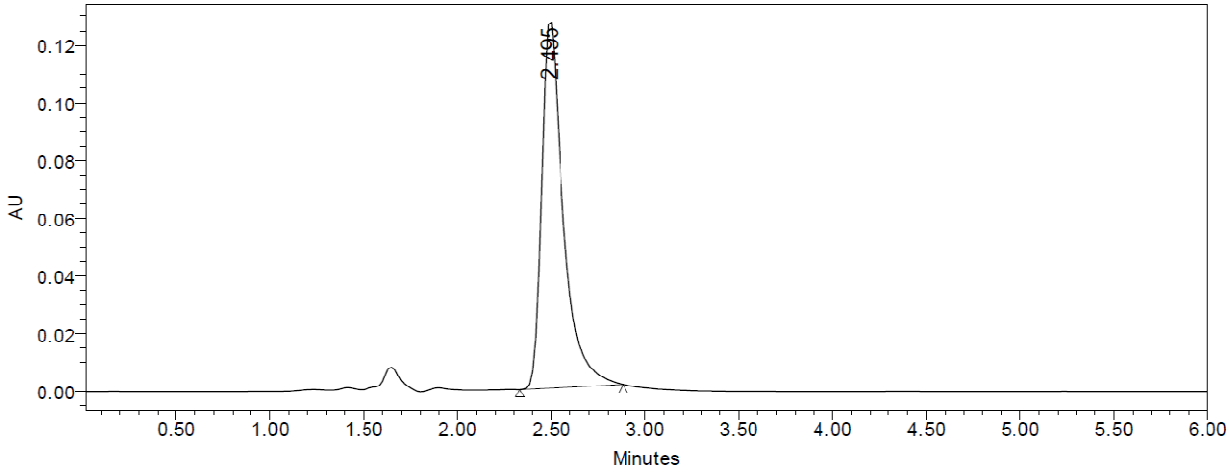

Figure 2: Typical chromatogram of lacosamide standard

Table 2: Linearity of lacosamide

Concentration (µg/mL)	Average area
5	253610
10	507214
15	770931
20	1035010
25	1293852
30	1570746
40	1998758
50	2511206

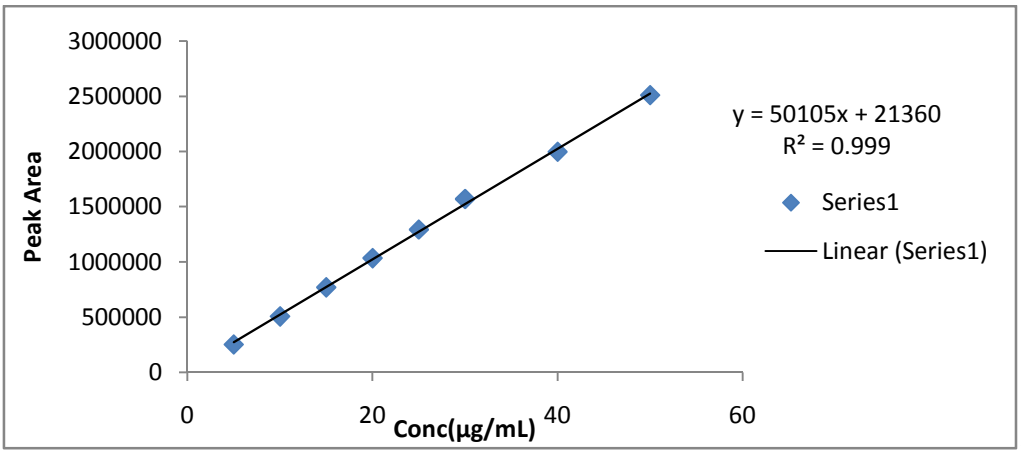

Fig 3: Linearity curve of lacosamide

Table 3: Precision of lacosamide

Injections	Area
1	1007697
2	1010004
3	1013740
4	1014100
5	1015397
6	1012342
Mean	1012213
SD	2874.75
% RSD	0.28

Precision

Precision of an analytical method is defined as the agreement between replicate measurements of the sample. Precision of the method was checked by injecting replicate injections of 20 µg/mL of the solution for six times and the response was recorded. It is expressed as the % relative standard deviation of the replicate measurements and presented in Table 3.

Accuracy

To determine the accuracy of the proposed method recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the standard samples to the pre-analyzed formulation within the linearity range. At each level, samples were injected in triplicate and the recovery percentage was determined and presented in Table 4.

Table 4: Accuracy studies of lacosamide

% Conc.	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	5.02±0.82	100.4 %	99.2%
100%	10.0	9.95±0.24	99.5%	
150%	15.0	14.65±0.76	97.7 %	

Specificity and selectivity

Specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in the formulation did not interfere with the drug peak and thus the method is specific for lacosamide. The HPLC chromatograms recorded for the drug matrix (mixture of drug and excipients) showed no interfering peaks within retention time ranges. Figure 2 and 4 shows the respective chromatograms of lacosamide were clearly separated which indicates the proposed method is selective.

Robustness

To determine the robustness of the method, parameters like flow rate, composition of mobile phase, detection wavelength and sonication time, were varied from the optimized chromatographic conditions. Solution was injected thrice in each of chromatographic condition. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in Table 5.

Table 5: Robustness studies of lacosamide

Parameters	Adjusted to	Average Area	R_t	SD	% RSD
Flow rate	0.8 mL/min	1035012	2.499	3928	0.38
	1.0mL/min	1014125	2.496	2583	0.25
	1.2ml/min	1132724	2.491	4137.5	0.37
Mobile phase composition	Acetonitrile : Buffer (35:65)	1015421	2.490	4542.8	0.45
	Acetonitrile : Buffer (40:60)	1012152	2.494	5167.2	0.51
	Acetonitrile : Buffer (45:55)	1036524	2.496	4315.4	0.42

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Limit of detection (LOD) and Limit of quantification (LOQ)

According to the determined signal-to-noise ratio, the LOD and LOQ of lacosamide obtained by the proposed method were 0.08µg/mL and 0.25µg/mL.

Table 6: System suitability of lacosamide

Parameters	Results of the method
Average area	1019362
Retention time(min)	2.496
Tailing Factor	1.6
Theoretical plates	2433.3
Plate per meter	16222
HETP	6.164×10^{-5}
Linearity range(µg/mL)	5-50
LOD(µg/mL)	0.08
LOQ(µg/mL)	0.25

System Suitability

System suitability tests were carried out on freshly prepared standard stock solutions of lacosamide by injecting standards in six replicates at 6 minutes interval and the values were recorded. System suitability parameters were shown in Table 6.

Assay of lacosamide tablet

Three different batches of Lacoset were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. Mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in Table 7. The results shown were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of the drug in pharmaceutical dosage forms. A typical chromatogram of lacosamide formulation shown in Figure 4.

Table 7: Contents of lacosamide in tablets

Sample tablet	Batch	Label claim(mg)	Amount found*(mg)±SD	%Amount found
Lacoset(50mg)	1	50	49.45±0.54	98.9
	2	50	49.26±0.65	98.5
	3	50	48.95±0.82	97.9

**Each value is an average of six replicates*

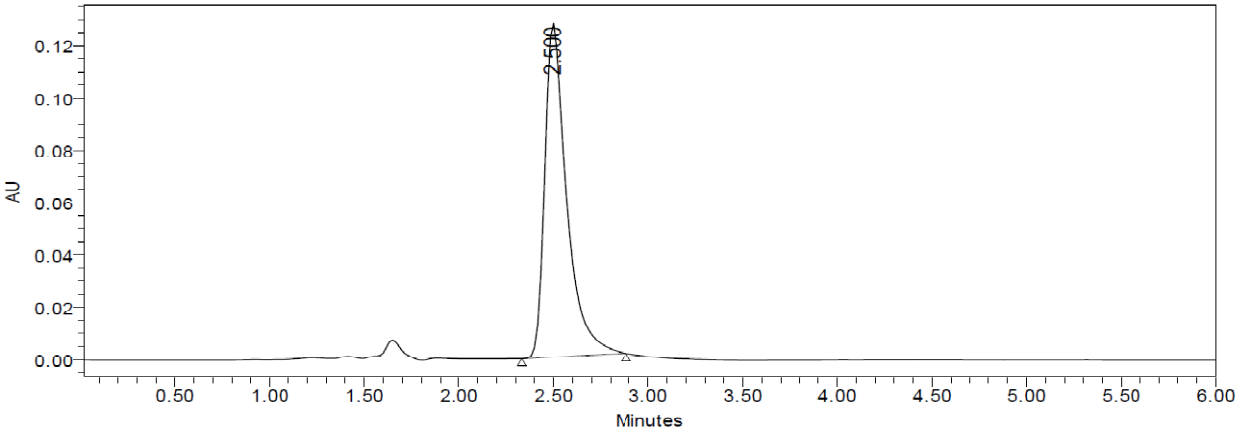

Figure 4: Typical chromatogram of lacosamide formulation

STABILITY STUDIES

A stress degradation study of lacosamide was performed using the validated method as per ICH guidelines [13]. Forced degradation for the drug was carried out under acid, base, thermal and oxidative stress condition. The results of analysis are then compared with freshly prepared samples and percent degradation was calculated.

Acid hydrolysis

1 mL of the stock solution (200µg/mL) was transferred to a 10mL volumetric flask. To which 1mL of 0.1M HCl was added and kept for 90 minutes at room temperature. Then it was neutralized with 0.1M NaOH and the volume was adjusted with mobile phase up to the mark. This solution was injected in to the HPLC system against a blank of HCl and mobile phase. The results were shown in Table 8 and Figure 5.

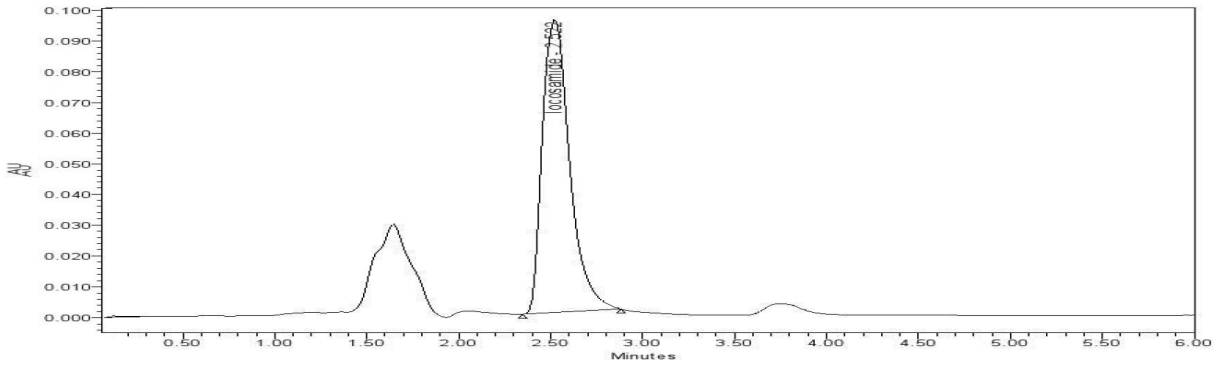

Figure 5: Chromatogram showing degradation of lacosamide in 0.1M HCl

Basic hydrolysis

1 mL of the stock solution (200µg/mL) was transferred to a 10mL volumetric flask. To which 1mL of 0.1M NaOH was added and kept for 90 minutes at room temperature. Then it was neutralized with 0.1M HCl and the volume was adjusted with mobile phase up to the mark. This solution was injected in to the HPLC system against a blank of NaOH and mobile phase. The results were shown in Table 8 and Figure 6.

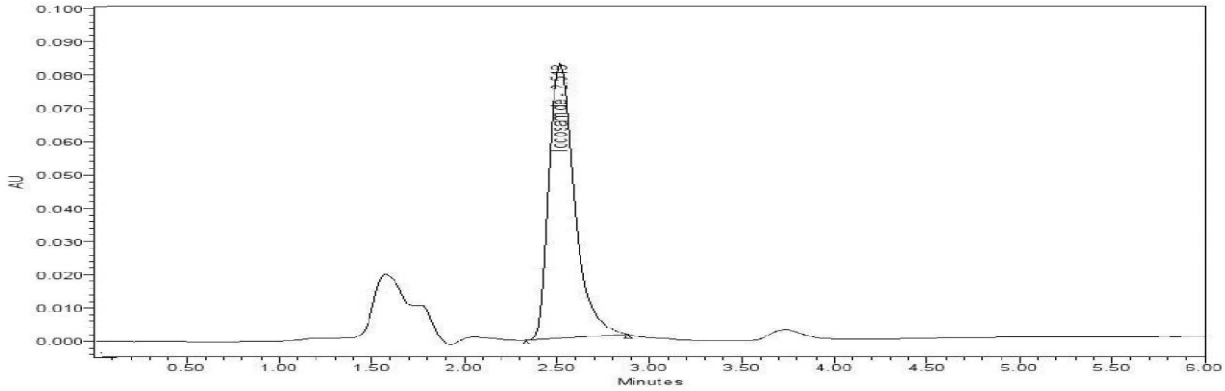

Figure 6: Chromatogram showing degradation of lacosamide in 0.1M NaOH

Thermal degradation

1 mL of the stock solution (200µg/mL) was transferred to a 10mL volumetric flask and the volume was made up to the mark with mobile phase. Then it was maintained at 50 °C for 90 minutes. This solution was injected in to the HPLC system against a blank of mobile phase. The results were shown in Table 8 and Figure 7.

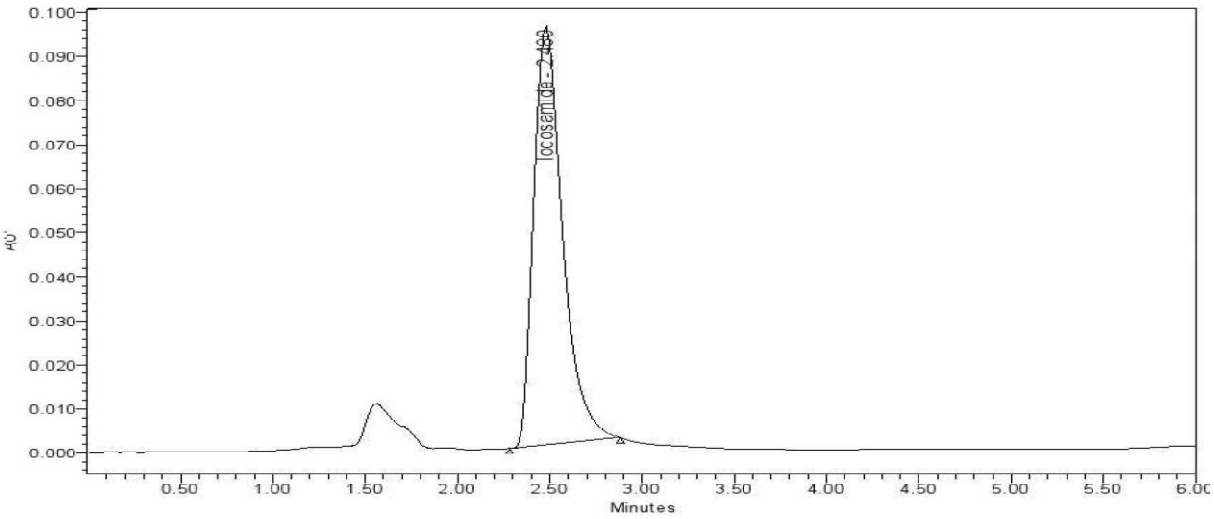

Figure 7: Chromatogram showing thermal degradation of lacosamide

Oxidation with H_2O_2 (3%)

1 mL of the stock solution (200µg/mL) was transferred to a 10 mL volumetric flask. To which 1mL of H_2O_2 (3%) was added and the volume was adjusted with mobile phase up to the mark and kept for 15 minutes at room temperature. This solution was injected in to the HPLC system against a blank of H_2O_2 and mobile phase. The results were shown in Table 8 and Figure 8.

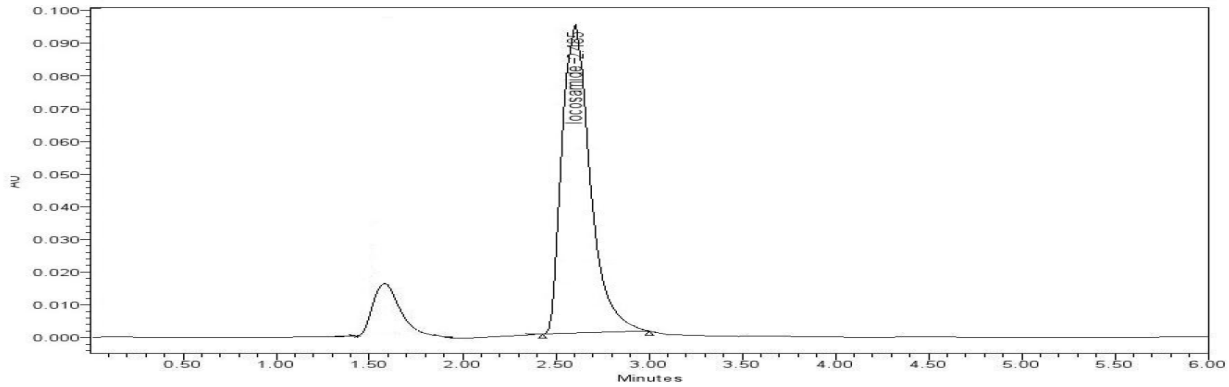

Figure 8: Chromatogram showing degradation of lacosamide in 3% H_2O_2

Table 8: Degradation studies of lacosamide

S. No.	Condition	Average area	%Degradation
1	Normal	1026031	-
2	Acid hydrolysis (0.1M HCl)	984985	4.01
3	Basic hydrolysis (0.1M NaOH)	886735	13.58
4	Thermal (50^{o}C)	1017256	0.86
5	Oxidation (3% H_2O_2)	1011667	1.4

RESULTS AND DISCUSSION

In this work a new sensitive, accurate, linear, precise and stability indicating RP-HPLC method has been developed for the estimation of lacosamide in bulk drug and pharmaceutical dosage form. A mixture of acetonitrile and phosphate buffer in the ratio of 40:60 v/v was found to be most suitable for the separation of lacosamide. The peak obtained was symmetrical and free from tailing. From the typical chromatogram the retention time was found to be 2.495 minutes. A good linear relationship (r^2 = 0.999) was observed in the concentration range of 5-50 µg/mL. Low values of standard deviation are indicative of high precision of the method. The assay of lacosamide tablet dosage form by the proposed method was found to be 98.5 percent. From the recovery studies it was found that about 99.2% of lacosamide was recovered, which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non interference of the excipients in the tablet dosage form. The result of the degradation studies indicated the specificity of the method. The study indicates that the developed method is simple, linear, accurate, specific and reproducible. Thus the developed method can be successfully used for the routine analysis of bulk drug and dosage forms of lacosamide within a short analysis time.

CONCLUSION

A stability indicating RP-HPLC method has been developed for the determination of lacosamide in tablet dosage form. The study indicates that the developed method is simple, linear, precise, accurate, specific and reproducible. Thus the developed method can be successfully used for the routine analysis of bulk drug and dosage forms of lacosamide within a short analysis time.

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