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Development and validation of a stability-indicating RP – HPLC method for determination of lacosamide

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ABSTRACT

A novel stability-indicating reverse phase high pressure liquid chromatographic assay method was developed and validated for the determination of lacosamide (LCM). This method was developed based on forced degradation data obtained by HPLC analysis. Lacosamide was subjected to stress under the conditions of hydrolysis (acidic, basic and neutral), oxidation, thermal and photolysis as approved by ICH. The separation of degradation products from lacosamide was accomplished on Hypersil BDS C_{18} Column using (250 x 4.6mm, 5µm) 0.01M mono basic potassium phosphate for adjusting the pH to 4.0 with orthophosphoric acid: acetonitrile (30:70, v/v) as mobile phase. The flow rate was 1.0 mL/min and the detection was carried out at 215 nm. The developed isocratic LC method was consequently validated for specificity, linearity, range, accuracy, precision and robustness. No previous reports were found in the literature regarding the degradation behavior of lacosamide.

Keywords: Lacosamide, RP-HPLC, Degradation products, Stability indicating.

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INTRODUCTION

Lacosamide newly licensed drug was used in the treatment of diabetic neuropathic pain and partial onset seizures in adults with epilepsy. It is a functionalized amino acid with a novel mechanism of action. It possesses excellent oral absorption, negligible protein binding, minimum interaction with other antiepileptic drugs and is excreted mainly in the urine. Epilepsy is a major neurological disorder, affecting up to 2% of the population worldwide and each year more than 100,000 new cases are diagnosed in US [1-7] and also number of cases found in India. Lacosamide drug was approved by United States Food and Drug Administration (FDA) in the year 2007.

The drug shows electrophysiological characters, modulates some voltage-gated sodium channels interacting with slow inactivated sodium channels and binding with collapsing response mediator protein (2) [8]. The chemical name of lacosamide is (2R)-2-(acetylamino)-N-benzyl-3-methoxypropanamide ($C_{13}H_{18}N_2O_3$) (Fig.1). Formulation of lacosamide was sold under the trade name 'Vimpat' for oral administration [9]. Recently, Clare et al. [10] have evaluated the suitability of set of commercial columns and found that Hypersil BDS C_{18} complies with system suitability.

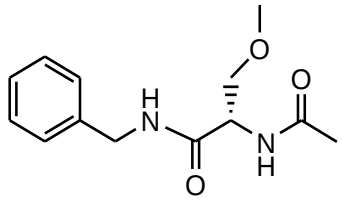

Fig. 1. Chemical structure of lacosamide

The objectives of the present manuscript describe the degradation behavior of lacosamide under hydrolysis (acid, base and neutral), oxidation, thermal and photolysis conditions. To optimize the liquid chromatography conditions to separate the drug from its degradation products on a reverse phase C_{18} column and to establish a validated stability-indicating assay HPLC method by UV detection at 215 nm. These studies provide precious information about drug's inherent stability and assist in the validation of analytical methods to be used in stability studies [10]. The developed HPLC Assay method was validated as per the International Conference on Harmonization (ICH) guidelines [12]. Furthermore, to the best of our knowledge, no stability-indicating assay method for this drug is reported in the literature.

MATERIALS AND METHODS

Experimental

Chemicals and reagents

Analytical grade reagents and HPLC grade solvents were used. Milli-Q water (Millipore Corporation, USA) was used. Acetonitrile and Methanol were purchased from Merck (Mumbai, India). Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid and hydrogen

peroxide were purchased from Sigma – Aldrich (Mumbai, India). Lacosamide drug was obtained as a gift sample from a local manufacturing unit in Hyderabad.

Instrumentation

The HPLC system consisting of two LC-20AT pumps, SPD- 20D UV detector, DGU-20A5 degasser (all from Shimadzu, Kyoto, Japan) were used. A reverse phase Hypersil BDS C_{18} column (250 x 4.6mm, 5µm) was used for separation of all the compounds. The chromatographic data were recorded using an HP- Vectra (Hewlett packed, Waldron, Germany) computer system with Spin Chrome data acquiring software (Shimadzu, Kyoto, Japan).

Degradation studies

All stress decomposition studies were performed at an initial drug concentration of 1.0 mg/ml. Each sample was dissolved in mobile phase and then added respective reagents (acid, base, water and hydrogen peroxide). The LCM was subjected to forced degradation under acidic, basic, and neutral conditions by refluxing in 0.5N HCl, 0.1N NaOH and distilled water at 70°C, respectively. Oxidative studies were carried out at room temperature (25±2°C) with 30% H_2O_2 for 12 h. The drug was placed in thermally controlled oven at 70°C for 3 days for thermal stress in solid and solution forms. For photolytic stress the drug was exposed to short wave UV light in UV chamber for 3days in solid and solution forms [13].

System suitability studies

System performance parameters of the developed HPLC method were determined by analyzing working standard solution. Chromatographic parameters, such as retention time (t_R), number of theoretical plates (N) and peak asymmetry (A_f) were calculated. The results are shown in Table 1.

Table 1: System suitability report

Compound (n=3).	t_R	A_f	N
Lacosamide	3.6	1.8	4533

n = 3 determinations, t_R = Retention time in minutes,
A_f = Asymmetry factor, N = No. of theoretical plates

Sample preparation

All the samples were prepared using mobile phase as diluent. The products, obtained from acid and base hydrolysis were neutralized with base and acid of same strength respectively. Neutral hydrolysis, thermal and photolytic samples were diluted by mobile phase to obtained 100 µg/mL solutions. The Oxidative stress sample was diluted by mobile phase to

obtained 10 µg/mL solution. All the prepared samples were filtered through 0.45µm nylon membrane.

RESULT AND DISCUSSION

Optimization of chromatographic conditions

During the optimization process, different conditions were imposed on Hypersil BDS C_{18} (250 x 4.6mm, 5µm) Column using 0.01M mono basic potassium phosphate with different pH values, with acetonitrile and methanol combinations as mobile phase. The base line as well as peak shapes were not good if methanol was used in mobile phase. Very good shape of lacosamide was observed by using acetonitrile and buffer mixture as mobile phase. To detect the drug and degradants with sufficient peak intensity, the wavelength 215 nm was selected. It was found that, at least 70% organic modifier was a requisite to elute all peaks within 10 min due to high lipopilicity. Neither buffer nor acetonitrile gave sufficient resolution alone to separate the degradation products from lacosamide. The injection volume was 20µl, mobile phase was at a flow rate of 1.0 ml/min and UV detection was at 215 nm. Finally 0.01M mono basic potassium phosphate adjusted to pH 4.0 with orthophosphoric acid: acetonitrile (30:70), which is an isocratic mode, gave good separation of the drug from its degradation products. The method was validated with respect to the parameters outlined in ICH guidelines [10].

Validation of the method

The objective of the validation procedure was to determine the specificity, linearity, accuracy, precision, detection, quantitative limits and robustness of the HPLC method and to assess the stability of the compound. The linearity was found in the concentration range of 50-150 µg/ml (r^2=0.9994) of the drug. The data from triplicate analysis of recovery showed that the % R.S.D. for each investigated concentration was <0.5%. The % R.S.D for intra- and inter-day precision at three different concentrations.,viz.,50, 100 and 150 µg/ml was <0.5%. The method was validated in accordance with ICH guidelines [14].

Accuracy

To assess accuracy, freshly prepared lacosamide drug was spiked in various amounts in the range of 50-150 µg/ml concentrations. Each solution was prepared in triplicate and injected in duplicate. The peak areas were used to calculate % recovery, mean, SD and %RSD. The recovery of the added drug was determined (Table 2).

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Table 2: Precision and recovery data

Precision data			
Conc., µg/ml	50	100	150
Intra- day precision			
Measured conc.,(µg/ml), ± S.D., R.S.D.(%)	49.7± 0.08, 0.42	99.8±0.11, 0.31	150.2±0.14, 0.20
Inter – day precision			
Measured conc.,(µg/ml), ± S.D., R.S.D.(%)	49.9±0.12, 0.53	100.2±0.09, 0.34	149.6±0.16, 0.41
Recovery data			
Conc., µg/ml	50	100	150
Calculated spiked conc.,(µg/ml) ± S.D., R.S.D.(%)	49.9±0.23,0.25	99.8±0.42, 0.53	149.6±0.40, 0.36
Recovery (%) n = 3 (replicates)	99.9	100.2	99.7

Precision

The Precision was evaluated in requisites of intra-day repeatability and inter-day reproducibility. The intra-day repeatability was investigated using 3 different sample solutions prepared (50, 100 and 150µg/mL). Each sample was injected in duplicates and the peak areas obtained were used to calculate % Assay, Mean, SD and %R.S.D values. The inter-day reproducibility experiment was repeated next day to establish its precision [15].

Linearity and range

The linearity of the method was evaluated at seven different concentrations of analytes within the range of 50 - 150µg/ml. These standard solutions were prepared by suitable dilution of stock solution with mobile phase. Each solution was analyzed in duplicate. Mean peak area values were plotted against concentrations of analytes. The linearity of the plot was evaluated using least squares linear regression analysis [Fig. 2][16&17].

For Lacosamide

$$Y = mx+c = 44.21x + 78.24, (r^2=0.9994)$$

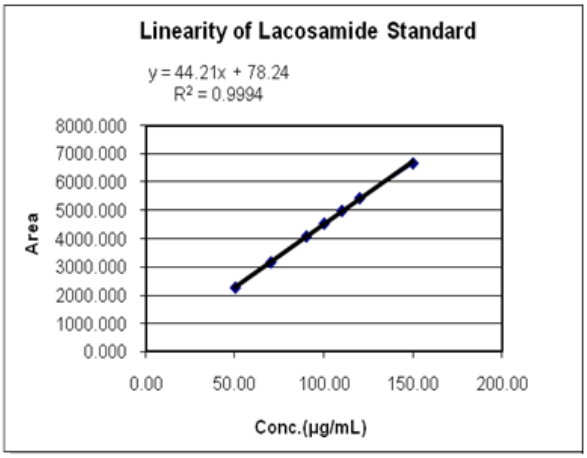

Fig. 2. Linearity of Lacosamide

Specificity and Selectivity

Specificity is the ability of the method to measure the analyte response in the presence of all the components that may be expected to present in the sample. The specificity of this LC method was assessed for lacosamide. A blank solution was injected and the chromatograms showed no inferring peaks at retention time of the drug. The resolution factors of the drug peak from the nearest resolving peak, and also among all other peaks.

LOD and LOQ

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. The drug substance to attain an average signal to-noise ratio of about 3:1

LOD = 3.3X standard deviation of y intercept /slope of calibration curve

Lacosamide - 2.8 µg/ml

The limit of Quantitation (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. The drug substance to attain an average signal to- noise ratio of about 10:1

LOQ = 10.1X standard deviation of y intercept /slope of calibration curve

Lacosamide - 8.9 µg/ml

Robustness

Robustness of the method was determined by making slight and deliberate changes in experimental conditions. The effect of change in flow rate (-10% to +10%), % of organic modifier in mobile phase (-2% to +2%) while the amounts of the other mobile phase components were held constant, column oven temperature (-2°C to +2C°), pH of the buffer (-0.2 units to +0.2 units) and the detection wavelength (-2 nm to +2nm) was studied. For all the above deliberately varied experimental conditions, there is no change in the chromatographic performance. It indicates the robustness of the method (Table 3).

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Table 3: Result of robustness study

Factor	Level	Mean % Assay, % R.S.D. of Results	
Flow rate (mL/min)	0.9	100.2,	0.41
	1.1	99.7,	0.23
Column oven temperature (°C)	23	99.5,	0.22
	25	100.1,	0.26
	27	99.8,	0.34
% of organic modifier	68	99.3,	0.18
	72	99.8,	0.21
Measurement Wavelength (nm)	213	99.9,	0.13
	217	100.3,	0.25
pH of the buffer (Units)	3.8	99.6,	0.32
	4.2	99.8,	0.24

Degradation of Lacosamide

Degradation behavior of lacosamide drug in various stress conditions was investigated by liquid chromatography. Typical chromatograms are shown in (Fig. 3a).

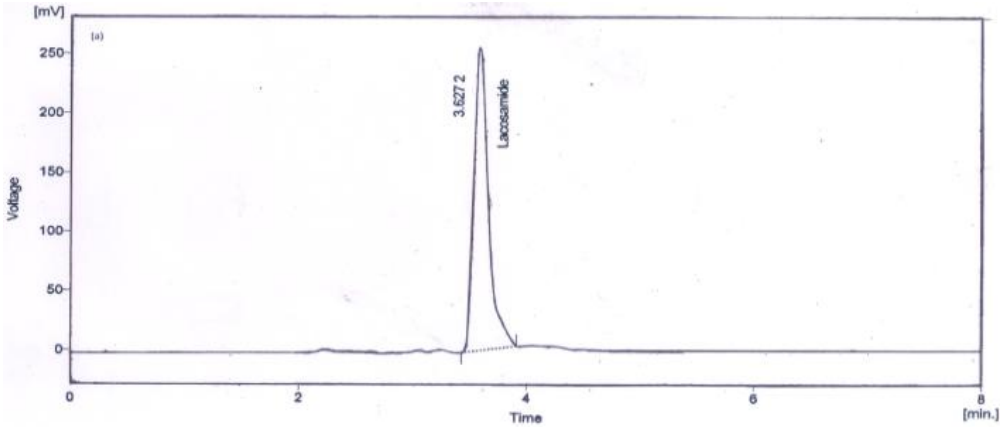

Fig. 3. (a) Lacosamide standard

Hydrolysis

Initially LCM was refluxed in 1.0 N HCl at 70ºC for 24 h and complete degradation of the drug was observed. Then the strength of the acid was reduced to 0.5 N HCl and refluxed at 70°C for 9h. Three degradation products (A1-A3) were formed on acid hydrolysis (Fig. 3b), three degradation products (B1-B3) on treatment of the drug with 0.1N NaOH for 24 h at 70ºC were formed (Fig. 3c). Under neutral hydrolysis, two degradation products were formed after 24 h at 70ºC. These products are N1-N2 (Fig. 3d)

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Fig. 3. (b) Acid degradation products

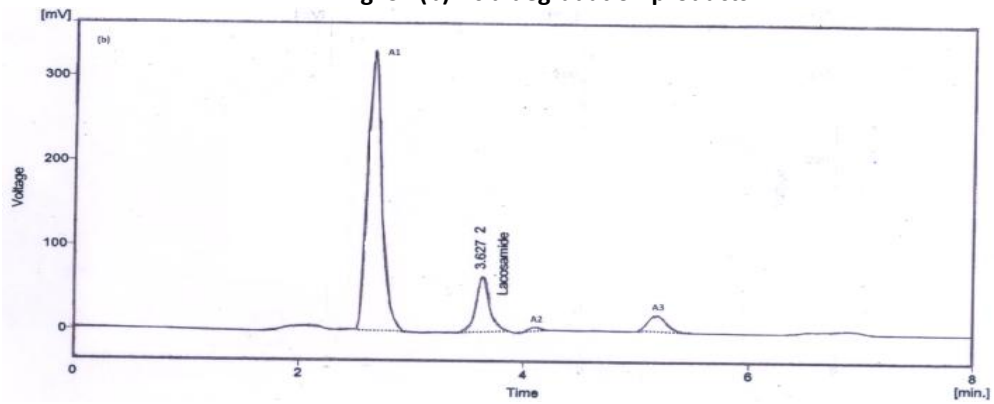


Fig. 3. (c) Base degradation products

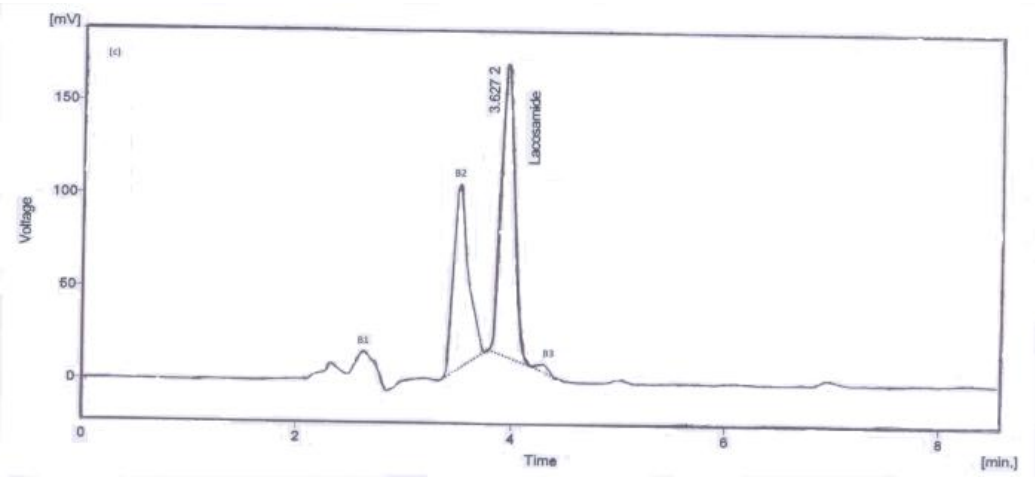
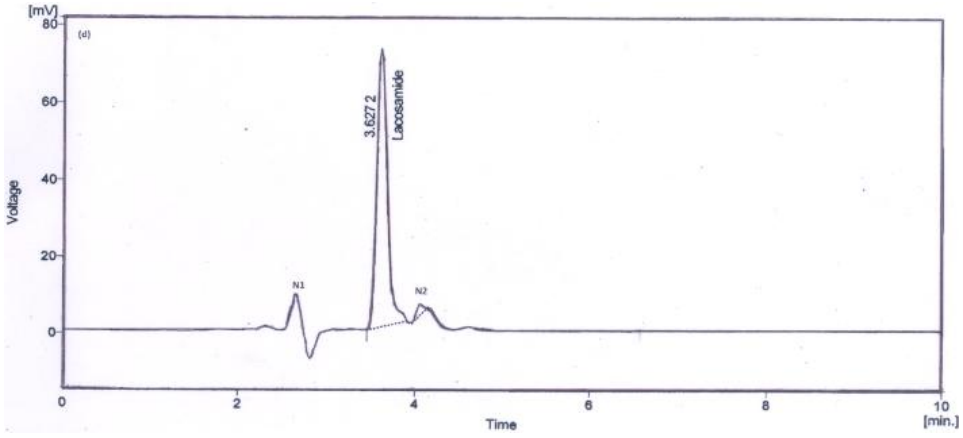


Fig. 3. (d) Neutral degradation products



Oxidation stress

Oxidative stress was carried out using 30% H_2O_2 at room temperature. Four degradation products H1-H4 were formed after 12 h. The four oxidative degradation products can be seen in Fig. 3e.

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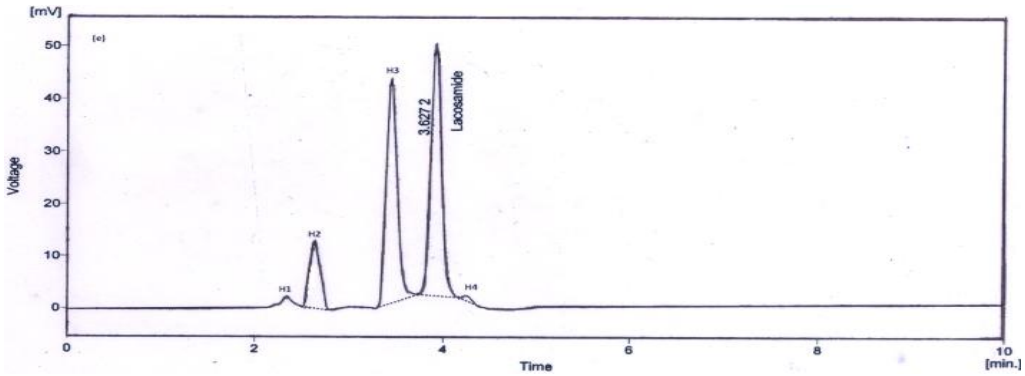
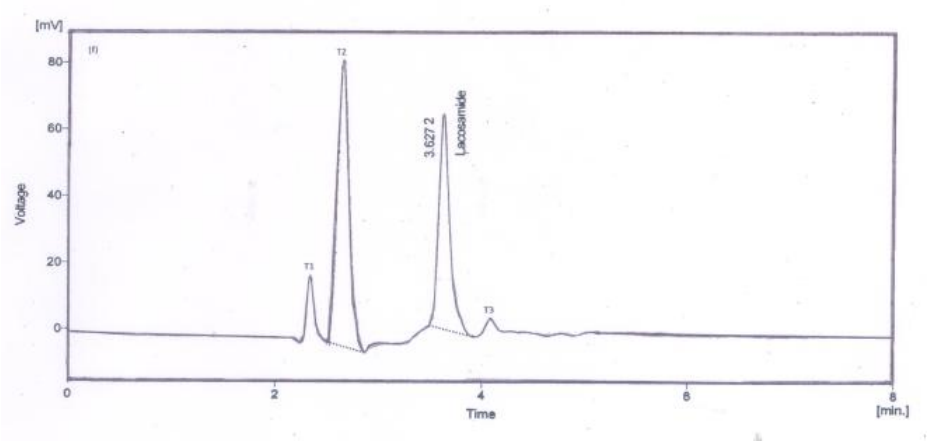
Fig. 3. (e) Peroxide degradation products



Thermal stress

The drug in solid form was kept at 70ºC for 3 days. No significant degradation was observed. However, the drug in solution three degradants (T1-T3) was formed. However thermal degradation was high under neutral hydrolysis (kept under reflux condition) when compared to thermal hydrolysis (kept in a thermally controlled oven) (Fig. 3f) [18].

Fig. 3. (f) Thermal degradation products



Photolysis

The lacosamide drug solution form was after exposure to short wave length(265nm) UV light. Two degradation products (P1-P2) one major and one minor degradants were formed. The photo degradation products were determined simultaneously by the previously described HPLC method (Fig. 3g).

Fig. 3. (g) Photolytic degradation products

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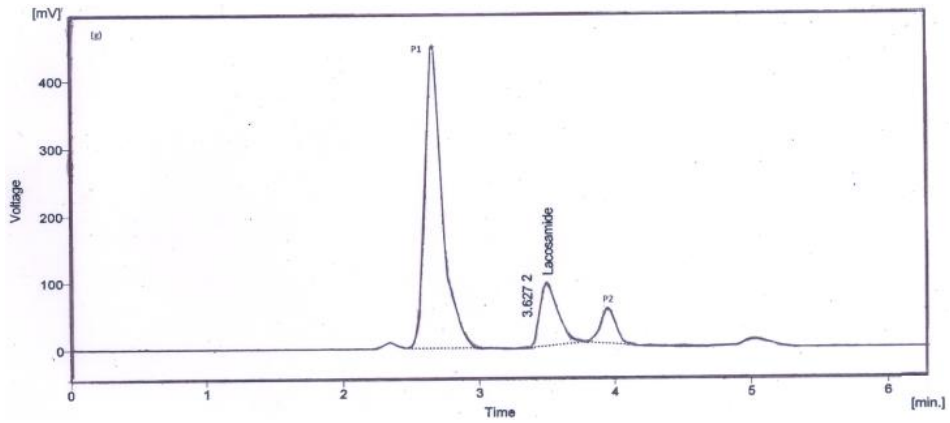

CONCLUSIONS

Forced degradation study on Lacosamide drug was performed under the conditions of hydrolysis (acidic, basic and neutral), oxidation, thermal and photolysis. Based on the information generated by forced degradation, a stability-indicating assay method was developed and validated. Linearity, precision, robustness, specificity, recovery data and stability indicators are excellent for this drug. The Quantitation, detection limits and accuracy are acceptable. This method can be used for analysis of lacosamide in stability samples.

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