Research Article

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

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ABSTRACT

An isocratic reverse phase High Performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Lacosamide in Bulk and its pharmaceutical formulation. Separation was achieved with Apollo Grace RP-18 ((Make: Waters Corporation; 250 mmx4.6 mm I.D; particle size 5µm)) Column and Sodium di-hydrogen ortho phosphate buffer (pH adjusted to 3.5 with diluted orthophosphoric acid): Acetonitrile (70:30) v/v as eluent at a flow rate of 1.0 ml/min. The Photo-Diode Array (PDA) was used for detection purpose and detection was performed at 215nm. The method is simple, rapid, and selective. The described method of Lacosamide is linear over a range of 5.0µg/ml to 15µg/ml. The correlation coefficient (r^2) was found to be 0.998. The method precision for the determination of assay was below 1.30%RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 99.1 to 101.5%. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of Lacosamide in bulk, and its dosage forms.

Keywords: Lacosamide, RP-HPLC, Validation, Dosage form.

INTRODUCTION

Lacosamide is a functionalized amino acid that has activity in the maximal electroshock seizure test, and is indicated for the adjunctive treatment of partial-onset seizures and diabetic neuropathic pain. Recent studies indicate that Lacosamide only affects those neurons which are depolarized or active for long periods of time, typical of neurons at the focus of an epileptic seizure, as opposed to other antiepileptic drugs such as carbamazepine or lamotrigine which slow the recovery from inactivation and reduce the ability of neurons to fire action potentials.

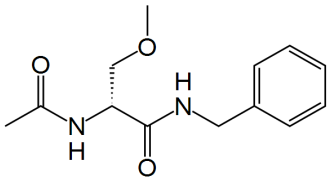

Figure 1: Chemical Structure of Lacosamide

For drug such as Lacosamide that is not intended to be absorbed into the bloodstream, Bioequivalence needs to be established by conducting a study with Pharmacodynamic endpoints. A comparison of In-Vitro dissolution profiles in different pH medium is an appropriate method for evaluating the bioequivalence of generic oral tablets.[1, 2]

MATERIALS AND METHODS

Instrumentation

The analysis of the drug was carried out on a Jasco LC system equipped with 2089 pump and photo-diode array detector (PDA) was used and a Reverse phase HPLC column Apollo Grace RP-C18 ((Make: Waters Corporation, Ireland); 250 mm x 4.6 mm I.D; particle size 5µm) was used. The output of signal was monitored and integrated using Chromnav software.

Chemicals and solvents

Milli-Q Water, Acetonitrile (ACN) (HPLC Grade), Orthophosphoric acid (GR Grade), Sodium dihydrogen ortho phosphate (GR Grade) was obtained from Merck, Mumbai.

Buffer preparation

Accurately weigh and transfer about 1.56 grams of Sodium di-hydrogen ortho phosphate in 1000 ml of purified water and mix. Adjust pH to 3.5 (±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 70:30 v/v respectively.

Standard preparation

Accurately weigh and transfer about 100mg of Lacosamide into a 100 ml volumetric flask, add 60 ml of Acetonitrile, sonicate to dissolve. Cool the solution to room temperature and dilute to volume with Acetonitrile. Transfer 1.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with Acetonitrile.

Sample preparation: (For Lacosamide Tablets 50mg)

Weigh and finely powder 20 Tablets. Accurately weigh and transfer equivalent to 10 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of Acetonitrile,

and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with ACN and mix. Filter the solution through 0.45µm membrane Filter. Transfer 1.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with ACN.

Chromatographic conditions

An Apollo Grace RP-C18 ((Make: Waters Corporation (Ireland); 250 mm x 4.6 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 20µl. The photodiode array detector was set to a wavelength of 215nm for the detection and Chromatographic runtime was 10 minutes.

RESULTS AND DISCUSSION

Method development

To develop a suitable and robust HPLC method for the determination of Lacosamide, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Thermosil RP-C18 ((Make: Waters Corporation (Ireland); 250 mm x 4.6 mm I.D; particle size 5µm)) with the following mobile phase. Accurately weigh and transfer about 1.56 grams of Sodium di-hydrogen phosphate monohydrate in 1000 ml of purified water and mix. Adjust pH to 3.5 (±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 50:50 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 60:40 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 70:30 v/v respectively as eluent at flow rate 1.0 ml/min. UV detection was performed at 215nm. The retention time of Lacosamide was about 5.6 minutes and the peak shape was good.

The chromatogram of Lacosamide standard using the proposed method and System suitability results of the method are given as follows. Lacosamide shows significant UV absorbance at Wavelength 215nm. Hence this wavelength has been chosen for detection in analysis of Lacosamide. [3, 4]

Table 1: System suitability parameters for Lacosamide by proposed method

Name of Compound	Number of Theoretical Plates	Retention time	Symmetry Factor
Lacosamide	11338	5.60	1.30

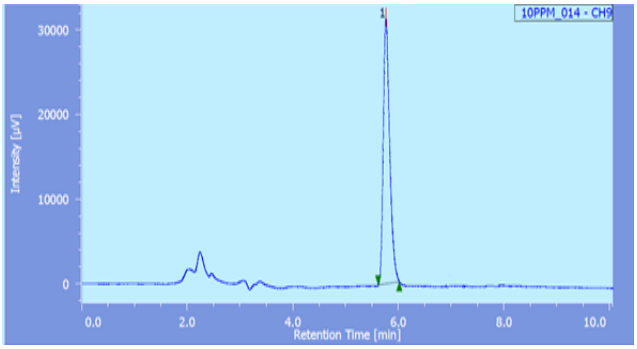

Figure 2: A typical HPLC Chromatogram showing the Peak of Lacosamide

Method validation [5, 6]

The developed RP-HPLC method extensively validated for the determination Lacosamide Content using the following Parameters.

Specificity Blank interference

A study to establish the interference of blank was conducted. Solvent 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.

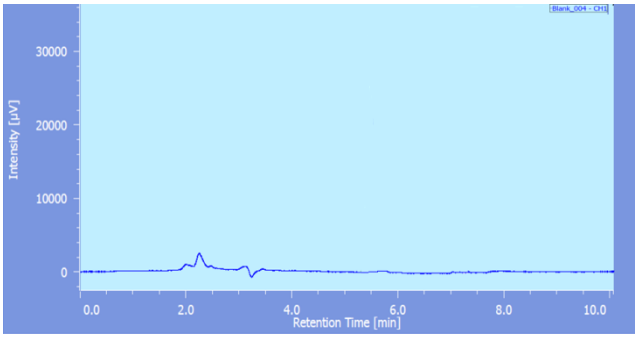

Figure 3: A typical HPLC Chromatogram showing the no interference of Solvent for Lacosamide

System and Method Precision

In the study of the instrumental system precision where, a RSD of 1.40% was obtained for the standard area obtained corresponding to the first day, being 1.37 % for the second day, respectively. The method precision study for six sample preparations in marketed samples showed a RSD of 0.5% and with the assay range of 99.1-101.5 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 1.30 % (For Standard). The same study was carried out for different analysts (*n* = 6 number of samples per analyst) obtaining a RSD of 1.65 % (Intermediate Precision) and with the assay range of

99.1-100.9 with an average of 99.5. The Overall %RSD for n=12 is 1.44. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Sir No.	8ppm	10ppm	12ppm
	(Conc in µg/ml) Day one		
1	227845	276253	346452
2	225621	275845	345214
3	230123	265451	352601
4	226541	278501	356201
5	223564	270012	345210
6	230012	275451	352012
	8.0	**10.0**	**12.0**
	Day Two		
1	223652	265241	356892
2	223021	270021	365210
3	230045	261247	370014
4	228545	270054	368214
5	228014	268511	365874
6	230016	274100	368994
Mean	**227215.50**	**268195.66**	**365866.33**
RSD	**1.37**	**1.65**	**1.30**

Table 2: Precision data table

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.

Linearity of detector response

The standard curve was obtained in the concentration range of 5-15µg/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.

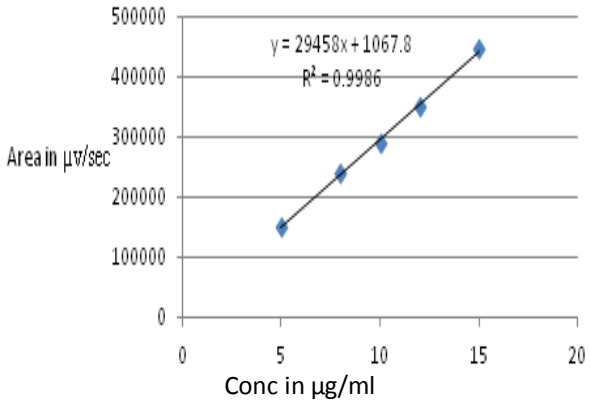

Figure 4: Calibration curve for Lacosamide

Table 4: Linearity Data

Parameter	Slope	Intercept	Correlation coeff (r^2)
	29458	1067.8	0.998

Forced Degradation:[7]

Acid Degradation Sample

Accurately weigh and transfer equivalent to 50 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of solvent, and sonicate for 10 minutes with intermittent shaking at controlled temperature. Then add 10ml of 0.1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 0.1N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent.

Base Degradation Sample

Accurately weigh and transfer equivalent to 100 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of solvent, and sonicate for 30 minutes with intermittent shaking at controlled temperature. Then add 10ml of 0.1N Base (NaOH), refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 0.1N Acid (HCl) and dilute to volume with diluent and mix. Filter the solution through 0.45µm membrane Filter. Transfer 1.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent.

Peroxide Degradation Sample

Accurately weigh and transfer equivalent to 10 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of solvent, and sonicate for 10minutes with intermittent shaking at controlled temperature. Then add 2ml of 5% Peroxide, refluxed for 30min at 60°C, then cooled to room temperature and dilute to volume with solvent and mix. Filter the solution through 0.45µm membrane Filter. Transfer 1.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with solvent.

Thermal Degradation Sample

Drug Powder exposed to heat at 105°C for about 5days. Accurately weigh and transfer equivalent to 10 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of solvent, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with solvent and mix.

Filter the solution through 0.45µm membrane Filter. Transfer 1.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with solvent.

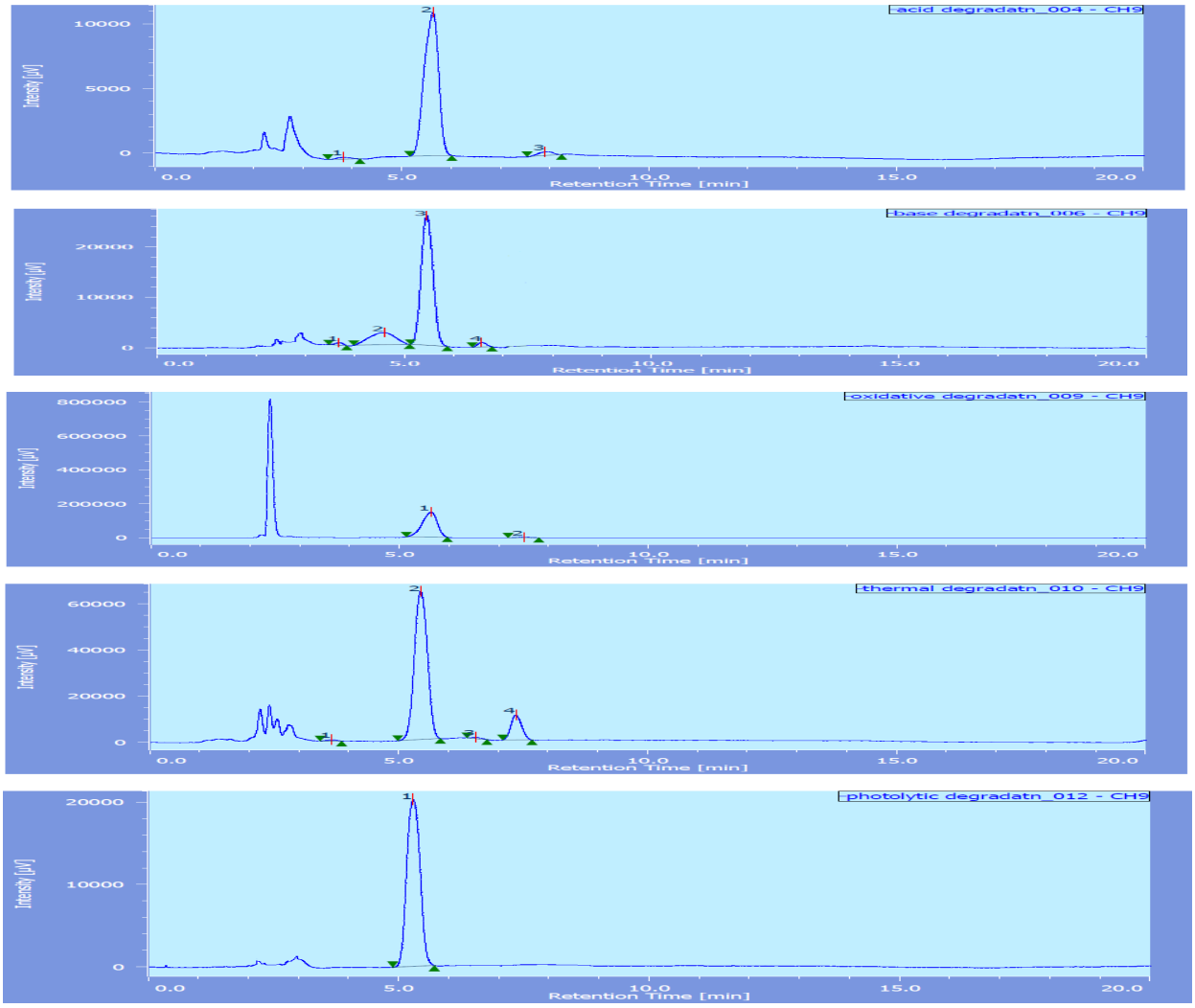

Figure 5: a) Acid Degradation, b) Base Degradation, c) Peroxide Degradation, d) Thermal Degradation, e) Photolytic Degradation

Table 3: Accuracy data

	Levels in µg/ml					Levels in µg/ml				
	5	**8.0**	**10.0**	**12.0**	**15.0**	**5**	**8.0**	**10.0**	**12.0**	**15.0**
Sir No	**Area**					**Recovery**				
1	151254	236002	290012	349578	447841	5.09	7.97	9.80	11.83	15.16
2	150652	237841	291021	358014	446201	5.07	8.03	9.84	12.11	15.11
3	151201	245874	290145	345210	447854	5.09	8.31	9.81	11.68	15.16
4	149501	238451	287541	352487	447496	5.03	8.05	9.72	11.92	15.15
5	151021	237410	291478	353320	448562	5.09	8.02	9.85	11.95	15.19
6	149521	238887	289140	350001	445784	5.03	8.07	9.77	11.84	15.09
Mean	**15525**	**239077.5**	**289889.5**	**351435**	**447289.66**	**5.07**	**8.07**	9.80	**11.89**	**15.14**
RSD	**0.5402**	**1.4536**	**0.4868**	**1.2218**	**0.2394**	**0.544**	**1.46**	**0.488**	**1.22**	**0.240**
Accuracy	**----**	**----**	**----**	**----**	**----**	**101.47**	**100.9**	**98.04**	**99.11**	**100.98**

Table 5: Forced Degradation Data

	Peak name	Rt	Area	NTP	Resolution	Symmetry
Acid	Degradant 1	3.80	2805	771	3.23	1.09
	Lacosamide	5.62	225721	1542	3.83	0.862
	Degradant 2	7.90	6162	2653	N/A	1.03
Base	Degradant 1	3.67	5643	2083	1.34	0.880
	Degradant 2	4.58	87685	314	1.16	0.903
	Lacosamide	5.45	427365	2330	2.83	1.07
	Degradant 3	6.56	10876	6087	2.19	1.05
Peroxide	Lacosamide	5.630	3094490	1582	3.627	0.830
	Degradant 1	7.503	69430	4107	N/A	0.958
Thermal	Degradant 1	3.65	7378	1477	4.088	0.981
	Lacosamide	5.44	116795	1924	2.604	1.019
	Degradant 2	6.54	7276	5515	2.140	1.185
	Degradant 3	7.35	162185	5268	N/A	1.110
Photolytic	Lacosamide	5.301	375750	1709	N/A	1.06

Photolytic Degradation

Drug Powder exposed to normal temperature for about 5days. Accurately weigh and transfer equivalent to 10 mg of Lacosamide into a 100 ml volumetric flask add about 70 ml of solvent, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with solvent and mix.

Filter the solution through 0.45µm membrane Filter. Transfer 1.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with solvent.

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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