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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF LACOSAMIDE IN BULK AND PARENTERAL DOSAGE FORM

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ABSTRACT

A simple, sensitive and precise RP-HPLC method was developed for the estimation of lacosamide in pure and injection form. The effective separation was achieved on a reverse phase Phenomenex C18 column using a mobile phase consisting of water and methanol in the ratio 40:60 v/v. The flow rate was 1.0 mL/min. The analyte was monitored using UV detector at 210 nm. The retention time was found to be 4.6 mins. The optimized method has been statistically validated for its sensitivity, linearity, precision, accuracy following the ICH guidelines. Thus the proposed method was successfully applied for the estimation of lacosamide in routine quality control analysis in bulk and its formulations.

Keywords: Lacosamide, Method development, Validation, RP-HPLC , injection.

INTRODUCTION

Lacosamide[1-2] chemically (R)-2-acetamido-N-benzyl 3 methoxypropionamide which was used for the treatment of diabetic neuropathic pain and partial onset seizures in adults with epilepsy. Lacosamide is a functionalized amino acid. Its molecular formula is C 13 H18 N2O3 and its molecular weight is 250.30. Lacosamide is a white to light yellow colour powder. It is sparingly soluble in water and slightly soluble in acetonitrile and ethanol. 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[3-11].

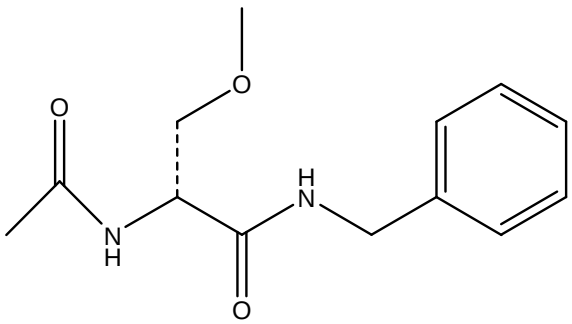

Fig. 1: Chemical structure of Lacosamide

Literature review reveals that few liquid chromatography procedures[12-19] have been reported for the determination of lacosamide in bulk and tablet dosage forms. To the best of our knowledge, no reports were found using economic mobile phase for the estimation of lacosamide by RP-HPLC –PDA method[23] for the estimation of lacosamide as per ICH guidelines[20-23].

MATERIALS AND METHODS

Lacosamide was supplied by Sun Pharma, Mumbai and lacosamide injection 200mg from local pharmacy was used and all the solvents, reagents are of HPLC grade (E.MERCK, Mumbai).The HPLC used was a Shimazdu's Prominence HPLC with LC-20AD series binary pump systems, SIL-20A HT Auto sampler, PDA SPD-M20A detector and DGU-20A degasser and LC Solutions software was used to acquire and process the data. A reverse phase Inertsil C18 (250 x 4.6mm, 5µm) analytical column was used.Chromatographic separation was achieved at an ambient temperature on a reversed phase column using a mobile phase consisting of a mixture of water and methanol in the ratio of (40:60 v/v). The mobile phase was filtered through 0.45µm nylon membrane filter and degassed by sonication for 2 min. Flow rate of 1.0 mL/ min was maintained and the detection was carried out at 210 nm. Separation was achieved on phenomenex C18 (250 x 4.6mm, 5µm) column with an injection volume of 10 µL.

Preparation of stock solutions

Weighed accurately and transferred 10 mg of lacosamide drug into a 10 mL volumetric flask, add about 5 mL of methanol as diluent, sonicate to dissolve the material completely, and made up to the mark.

Preparation of sample solutions

In a 10mL volumetric flask 5mL of methanol was added and 1mL of lacosamide injection(200mg/20mL) solution was mixed well. Then the remaining amount of methanol was added up to the mark. The sample solution was sonicated for 2 mins.

METHOD VALIDATION

Linearity

A linear relationship should be evaluated across the range of analytical procedure which may be demonstrated directly on the drug substance by dilution of a standard stock solution. The linearity of Lacosamide responses were determined by preparing and injecting standard solutions in the range of 10-50µg/mL. The data was given in Table 1.

System Precision

Repeatability of standard application was carried out using six replicates of the same standard concentration (30µg/mL). The data was given in Table 3.

Method Precision

The method precision was determined by preparing a sample solution of Lacosamide injection. Repeatability was carried out using six replicates of the same concentration (30µg/mL). The data was given in Table 2.

Accuracy

The accuracy of the method was determined through recovery studies by the standard addition method by spiking 80%, 100%, 120% of the known quantities of standard within the range of linearity to the solution of drug product with 30 µg/mL of Lacosamide solution was analyzed in triplicate, the data was given in Table 4.

LOD and LOQ

LOD and LOQ were determined by calibration curve method. Standard solution of Lacosamide was prepared in the range of 10-50µg/mL and injected (10 µL) in triplicate. LOD and LOQ were calculated by using following equations: LOD = (3.3 × σ)/s; LOQ= (10.0× σ)/s (Where, σ is the standard deviation of Y-intercept of regression lines s is mean of the slopes of the regression lines).

System Suitability

System suitability studies were carried out by injecting a 30µg/mL standard of Lacosamideat different injection volumes. The data was given in Table 5.

Specificity

Specificity studies were carried for both pure drug and drug product by comparing the 3D plots with blank (diluent) and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is no attributable to more than one component as the impurities are not available by analyzing the peak purity index data. The data was shown in Fig 3 and 4.

Assay for marketed formulation

The proposed validated method was successfully applied to determine the Lacosamide in parenteral dosage form without the interference of excipients. 10 µL of sample solution was injected. The assay was repeated for six times and the amount of the drug present in injection was estimated from calibration equation. The mean % recovery was determined. The data was shown in Table No:6.

RESULTS AND DISCUSSION

The present investigation was carried out with a view to develop a rapid and economical RP-HPLC method for the estimation of Lacosamide in bulk and parenteral dosage forms. In the present investigation, different mobile phase combinations were tested to develop a highly economic LC method, for the

analysis of Lacosamide in bulk and parenteral formulations. Initial trials were carried with PhenomenexC18 column (250x4.6mm, 5µm) using water and methanol as mobile phase in different ratios with 1.0mL/min flow rate with water as a diluentThe trials were continued by changing the mobile phase composition and optimized to water and methanol(40:60v/v). Under these conditions the Lacosamidewas eluted at 4.6mins along with the solvent front, theoretical plates and tailing factor was found to be within the limits.

The linearity was established with in concentration range of 10-50µg/mL with a good correlation coefficient of 0.995. The data was shown in fig-2. Precision studies was found to be good as percent RSD less than 2. The analyte peak is evaluated by 3D plot to confirm the existence of only lacosamide at 4.6mins. The recovery of the added standard to the drug product was found to be in the range of 98.96-100.43%.LOD and LOQ was found to be 0.2µg/mL and 0.8µg/mL respectively. The specificity of the method was established by spiking with diluent solution of commonly used excipients in the injection and showed no peak within the retention time of drug and also over the range of 8.0mins. The method was found to be robust with in deliberate variations like flow rate and wavelength.

CONCLUSION

The proposed RP-HPLC method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of Lacosamide using binary mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of Lacosamide without interference from diluent and placebo. The proposed method is highly sensitive, economical, reproducible, reliable, rapid, specific and robust.

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Table 1: Linearity of lacosamide (n=2)

Concentration (µg/mL)	Avg Area of the peak
10	261776
20	463042
30	703977
40	964198
50	1238865

Table 2: Method Precision of Lacosamide

Injections	Area
1	780184
2	783191
3	788870
4	778177
5	787822
6	790134
Mean	784729.7
Standard Deviation	4937.2
%RSD	0.62

Table 3: System Precision of Lacosamide

Injections	Area
1	699464
2	704092
3	699050
4	687446
5	703987
6	692895
Mean	697822.3
Standard Deviation	6533.926
%RSD	0.936

Table 4: Accuracy of Lacosamide (n=3)

% Concentration	Amount Added	Amount found	% Recovery	%RSD
80	24	23.41	98.96	0.518
100	30	30.11	100.38	1.231
120	36	36.15	100.43	0.283

Table 5: System suitability of Lacosamide

Injection volume (µL)	Retention time (min)	Tailing factor	Theoretical plates (N)
10	4.62	1.453	6442.7
20	4.62	1.480	6424
30	4.62	1.496	6503
40	4.62	1.498	6545
50	4.62	1.525	6492
mean	4.622	1.4904	6481.2
% rsd< 2	0.096	1.744	0.75
Limits		**< 2**	**> 2000**

Table 6: Assay of Lacosamide (n=3)

Concentration (µg/mL)	Area	% content	Amount(mg)
30	780184	110.8	33.24
30	783191	111.23	33.37
30	788870	112.04	33.61
Mean		111.356	33.40
%RSD		0.565	0.561

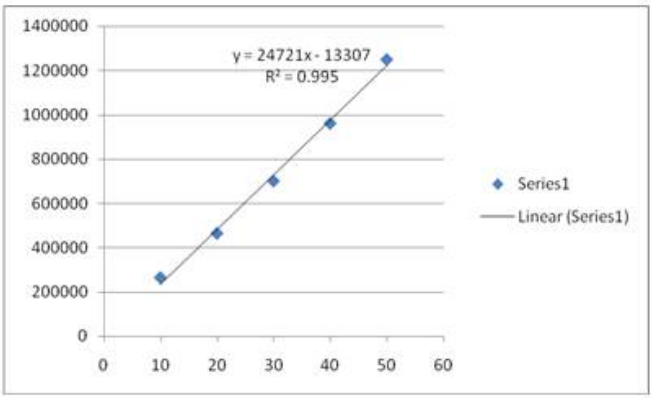

Fig. 2: Linearity Curve of Lacosamide

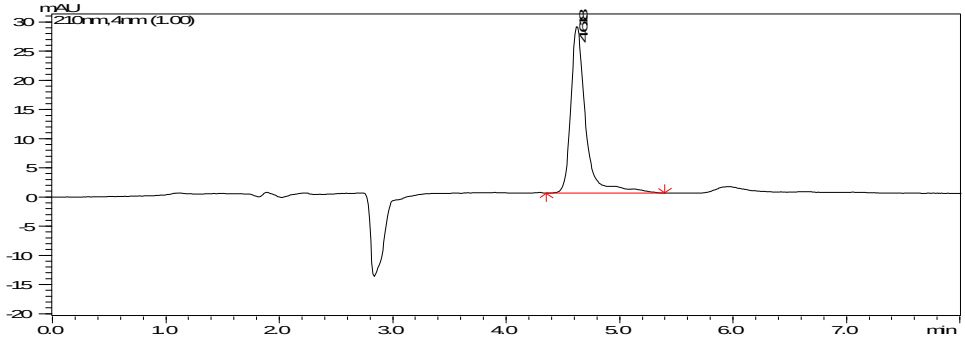

Fig. 3: Chromatogram of Lacosamide Standard and assay

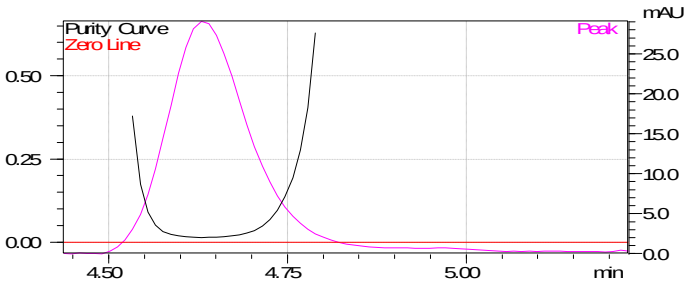

Fig. 4: Peak purity plot for lacosamide

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