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METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF LACOSAMIDE AND ITS RELATED SUBSTANCES IN PHARMACEUTICAL FORMULATION BY RP-HPLC

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ABSTRACT

A simple, sensitive, and precise high performance liquid chromatographic method for the determination of related substances of Lacosamide in pharmaceutical formulation has been developed and validated. The Impurities were well separated on a Inertsustain HP C18 (100mm X 4.6mm, 3µm) by the gradient program using Phosphate buffer (pH 2.0) and Acetonitrile at a flow rate of 1.0 mL /min with detection wavelength at 210 nm. The method is considered 'accurate' if the individual % recovery of Lacosamide and its impurities at each level should not be less than 90.0% and should not be more than 110.0%.

Keywords: Lacosamide, HPLC, Related substances, Method Development.

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INTRODUCTION

Lacosamide 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. 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 (1). The chemical name of lacosamide is (*R*)-2-(acetylamino) -*N*-benzyl-3-methoxypropanamide ($\mathbf{C_{13}H_{18}N_2O_3}$).

The literature survey reveals that there are available HPLC Methods, UV Spectroscopic methods for assay of Lacosamide. Furthermore, to the best of our knowledge; no stability-indicating HPLC method is reported in the literature for related substances of Lacosamide injection (2-10).

The objective of the present work is to develop a validating method for analysis of Lacosamide related substances in the Lacosamide formulation.

MATERIALS AND METHODS

Acetonitrile and Potassium dihydrogen phosphate from Merck PVT Ltd, Mumbai. VIMPAT (Lacosamide). Injection 200 mg/20 mL Injection was obtained from UCB Pharma, SA.

Optimized chromatographic conditions

Separation was carried out using Column- Inertsustain HP C18 (100 mm x 4.6 mm, 3 µm), Buffer preparation: 1.36 g of potassium dihydrogen phosphate in 1000 ml water, Buffer :10 mM Potassium Phosphate Buffer pH 2.0, Organic solvents : Acetonitrile, Mobile phase - A : pH 2.0 Buffer: Acetonitile – (ratio 98:2), Mobile phase - B: Acetonitrile: Water (ratio 60:40), Diluent : Water and Acetonitrile (90:10), Mode : Gradient, Flow Rat : 1.5 mL/min, Injection Volume : 10 µL, Wave Length : 210 nm, Detector : UV-detector.

Preparation of solutions

Preparation of diluted standard solution

50 mg of Lacosamide working standard was transferred in 100 ml volumetric flask and then 35ml of diluent was added, sonicate to dissolve and make up volume with diluent. Pipette out 5ml of above solution and transferred into 100ml volumetric flask and adjusted the volume with diluent and mix well. Further transfer 4ml of above solution and transferred into 100ml volumetric flask and adjusted the volume with diluent and mix well (11).

Preparation of test solution

Pipette 5mL of Lacosamide injection into a 100 ml volumetric flask and then added 70 ml diluents shake well and adjusted the final volume with diluent and mixed.

Buffer preparation

1.36 gm of potassium dihydrogen phosphate in 1000ml of water and P^H was adjusted to 2.0 with orthophosphoric acid and filtered through 0.45 µm membrane filter.

Mobile Phase

Mobile phase-A: pH 2.00 KH_2PO_4 buffer: Acetonitrile (980:20 v/v), Mobile phase - B: Acetonitrile : Water (60:40 v/v)

Preparation of Diluent

Mixture of Water and Acetonitile (90:10 v/v) respectively.

Method Validation (11, 12)

The proposed method was validated for parameters such as

Quantification: Equal volumes, (1µL), of the standard preparations and the test preparations that contain Lacosamide in the diluent were injected into the chromatograph and the quantified for Lacosamide and its impurities.

Linearity, Limit of detection and limit of quantification: Calibration graphs were constructed for Lacosamide and its impurities in either standard solution .The degree of linearity was assessed by the correlation coefficient, y-intercept, and slope. The limit of detection, LOD and the limit of quantitation LOQ have been estimated as 3 S.D. and 10 S.D. of the y intercept and slope.

Precision: The precision was performed by preparing six individual preparations as per the method of analysis and evaluated for percentage of Lacosamide and its individual impurities and percentage of total impurities.

Accuracy: The samples were prepared by spiking the active substances and impurities stock solutions into the drug placebo mixture and the percent recovery was estimated.

Solution stability: The solutions prepared was tested at initial, 24hrs and 48Hrs by maintaining at room temperature and estimated for Lacosamide impurity content.

Robustness: Robustness was conducted by making the variations in flow rate, Column oven temperature and percentage of Acetonitrile.

Ruggedness: The prepared solutions were filtered through 0.45 µ PVDF syringe filter and 0.45 µ PTFE syringe filter and evaluated difference between the Lacosamide and impurities content.

Intermediate precision: The test was performed with another analyst on different day, different system and different column and the Lacosamide and its impurity contents were reported.

RESULTS AND DISCUSSION

For RP-HPLC method pH 2.00 KH_2PO_4 buffer and Acetonitrile (98:2 v/v) as mobile phase A and Acetonitrile: water (60:40 v/v) was selected as a mobile phase-B which gives good resolution and good peak shapes for Lacosamide and their related substances. The flow rate was set at 1.5 mL/min, and the detection was carried out with UV detector at 210 nm, Inertsustain HP C18 column, 100 × 4.6 mm, 3 µm columns was used for the separation (Fig-1). The results of analysed impurities were given in Table-1.

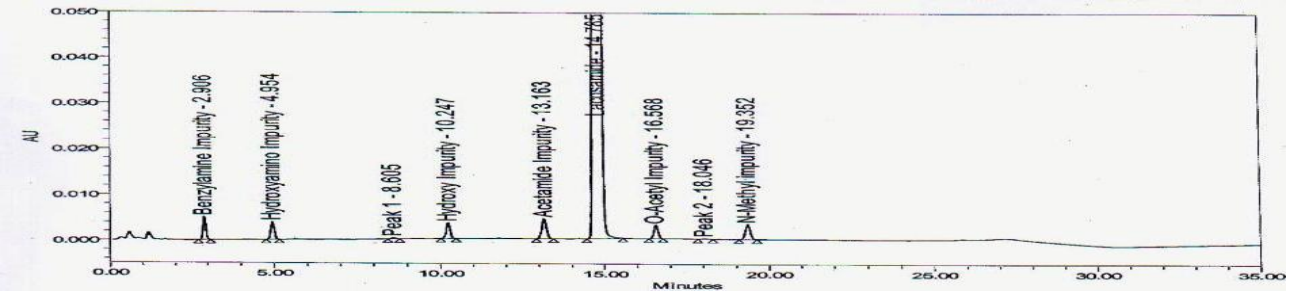

Fig-1 Chromatogram for sample solution by optimized method

Table- 1 Results for Assay of Lacosamide impurities

S.NO	Name of Impurity	Area	RRT	% Impurity	Limits
1	Benzyl Amine Impurity	749365	0.19	0.17	known Impurities NMT-0.2%
2	Hydroxy Amino Impurity	624745	0.33	0.15	
3	Hydroxy Impurity	756538	0.69	0.13	
4	Acetamide Impurity	985248	0.89	0.19	
5	O-Acetyl Impurity	518461	1.12	0.11	
6	N-Methyl Impurity	1018715	1.31	0.19	
7	Peak 1	44501	0.58	0.02	Unknown Impurities NMT-0.2%
8	Peak 2	26346	1.22	0.01	
	Total			0.97	Total impurities NLT 2.0%

The *Correlation coefficient* for Lacosamide, benzylamine, hydroxylamine, hydroxyl, acetamide, O-Acetyl, N-Methyl impurities was found to be 0.9999, 0.9999, 0.9998, 0.9999, 0.9998, 0.9999 and 0.9997 respectively, which indicates that the peak responses are linear. This concluded that the method was linear throughout the range selected (Fig-2-8).

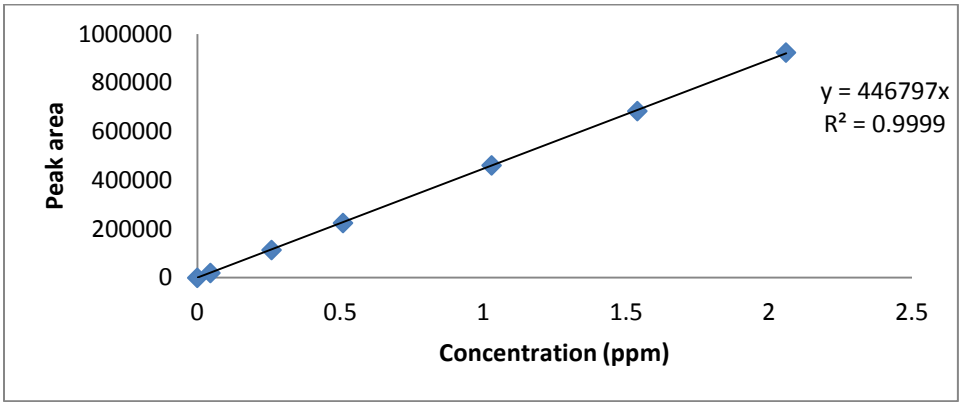

Fig-2 Linearity Graph of Lacosamide

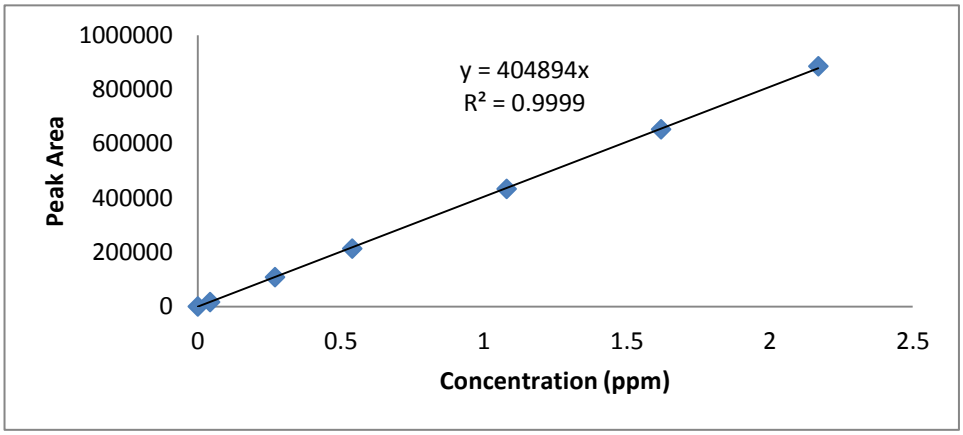

Fig-3 Linearity Graph of Benzyl amine Impurity

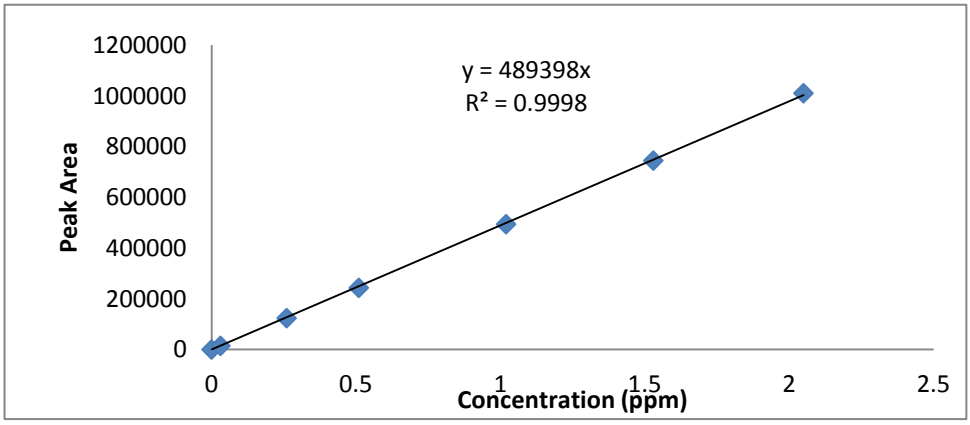

Fig-4 Linearity Graph of Hydroxy Amine Impurity

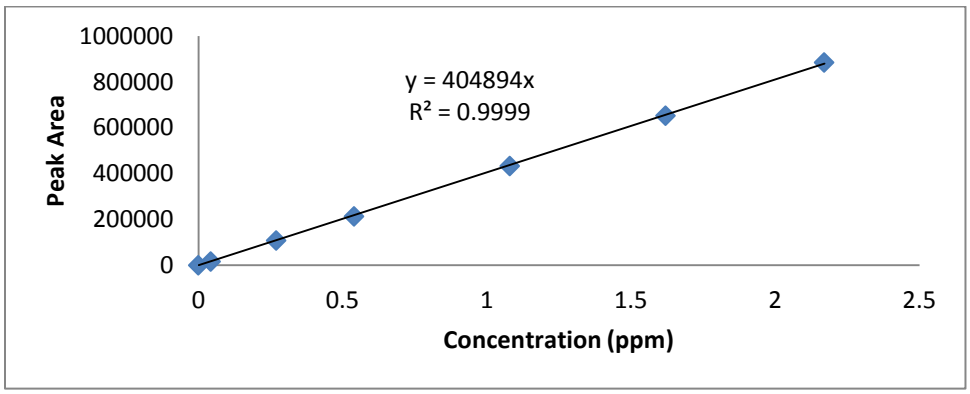

Fig-5 Linearity Graph of Hydroxy Impurity

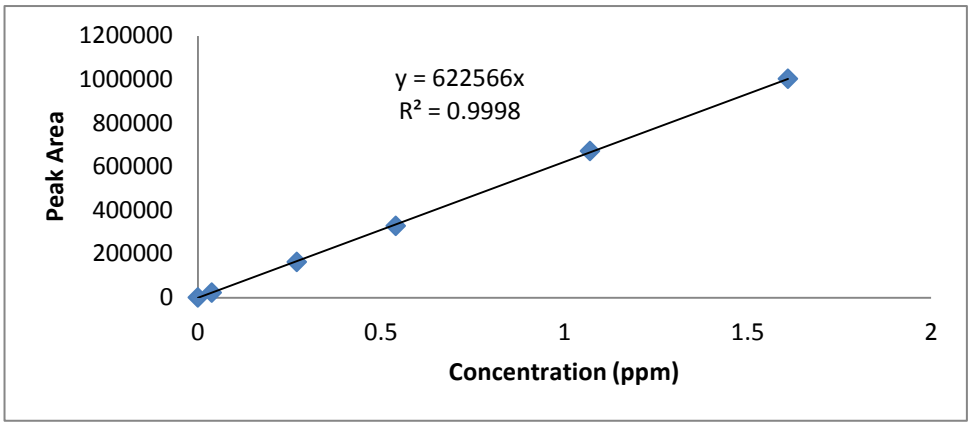

Fig-6 Linearity Graph of Acetamide Amine Impurity

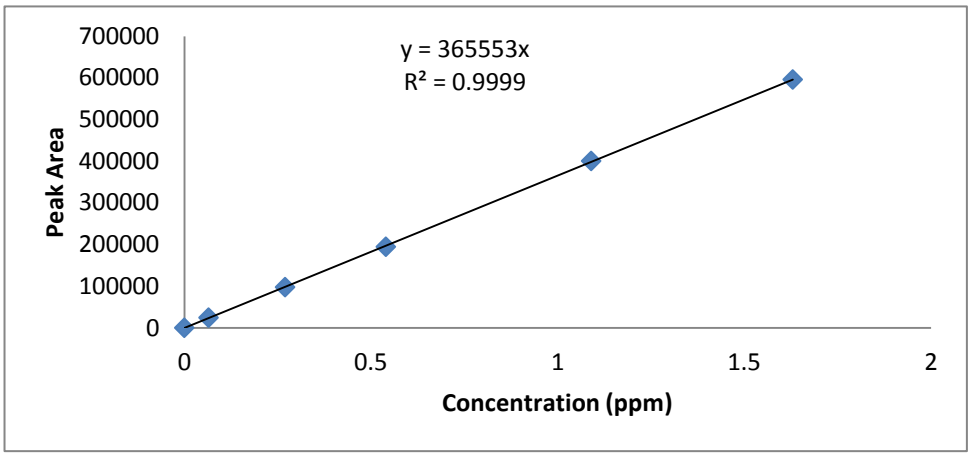

Fig- 7 Linearity Graph of O-Acetyl Impurity

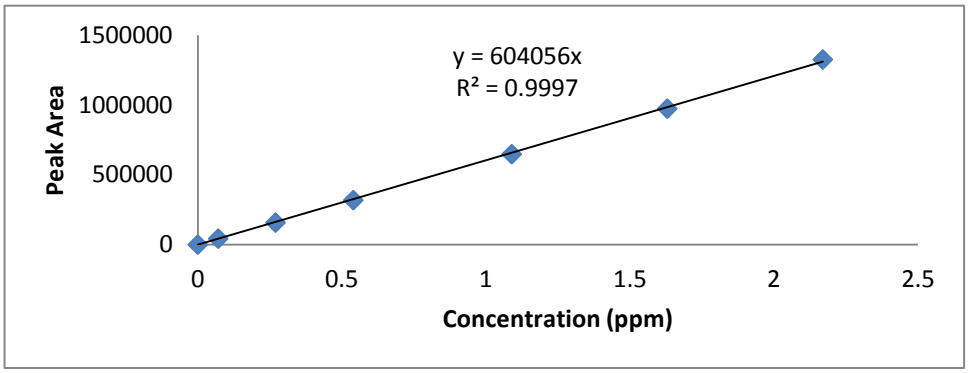

Fig- 8 Linearity Graph of N-Methyl Impurity

The LOD and LOQ of the Lacosamide impurities were according the ICH guidelines. The results of LOD and LOQ of Lacosamide impurities were given in Table-2

Table-2 Results of LOD & LOQ of Lacosamide impurities

Name of the Compound	LOD			LOQ		
	Concentration (ppm)	*Area*	S/N Ratio	Concentration (ppm)	*Area*	S/N Ratio
Lacosamide	0.015	6607	2.43	0.046	19820	9.90
Benzyl Amine Impurity	0.009	4659	2.38	0.027	14337	10.21
Hydroxy Amino Impurity	0.010	4780	2.51	0.031	14342	10.17
Hydroxy Impurity	0.014	5782	2.59	0.043	16236	10.10
Acetamide Impurity	0.012	7824	2.41	0.038	22104	10.13
O-Acetyl Impurity	0.021	8265	2.30	0.065	24420	10.21
N-Methyl Impurity	0.023	14124	2.36	0.071	42724	10.07

The developed method was validated for specificity, accuracy, precision, recovery, linearity, robustness, ruggedness and system suitability. The percentage of recovery of Lacosamide was found to be 96.92% at 100% level their related substances was found to be within acceptance range at 100% level. The low standard deviation values and good recoveries indicate the reproducibility and accuracy of the developed method. As well the % RSD values for precision study also were within acceptable limit**.** Slight changes in the experimental conditions did not affect significantly the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method.

CONCLUSION

An HPLC method for related compounds in the commercial drug products and in the injection formulation was validated in this study. Lacosamide and its impurities which may co exist with it as impurities or as degradants gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability. All the statistical values (% recovery, RSD, the slope and the intercept, LOD and LOQ) calculated were within the acceptable

limits. It can be used for estimation of Lacosamide and its related substances in bulk drugs, liquid dosage form and quality control purposes.

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