WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 11, 2546-2558. Review Article ISSN 2277–7105

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF LACOSAMIDE AND ITS RELATED SUBSTANCES IN PHARMACEUTICAL FORMULATION BY RP-HPLC

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Article Received on 05 May 2026,
Article Revised on 25 May 2026,
Article Published on 03 June 2026,

https://doi.org/10.5281/zenodo.20537056

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How to cite this Article: Dr. Ch. Saibabu*[1], K. Bhargavi[2], Dr. M. Bhaskar[3] (2026). Stability Indicating Method Development And Validation Of Lacosamide And Its Related Substances In Pharmaceutical Formulation By Rp-Hplc World Journal of Pharmaceutical Research, 15(11), 2546-2558.

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, validated and used for the determination of related substances in commercial pharmaceutical products. The Impurities were well separated on a Inertsustain HP C18 (100mm X 4.6mm, 3µm) by the gradient program using method pH 2.00 KH_2PO_4 buffer: Acetonitrile (98:2 v/v) as mobile phase-A and Acetonitrile: water (60:40 v/v) as a mobile phase-B which gives good resolution and good peak shapes for Lacosamide and their related substances at a flow rate of 1.5 mL/min with detection wavelength at 210 nm. Calibration curves were found linear with correlation coefficient of 0.999 with a linearity range LOQ -200%. 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. The method was found to be stable with no interference with degradation products.

KEYWORDS: Lacosamide, HPLC, Related substances, Method development.

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-(acetyl amino)-N-benzyl-3-methoxypropanamide (C13H18N2O3). The literature survey reveals that there are available HPLC Methods,[9-16] UV Spectroscopic methods,[15-17] Furthermore, to the best of our knowledge; no stability-indicating UPLC method is reported in the literature. The objectives of the present manuscript describe the degradation behaviour 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 HSS, C18 column and to establish a validated stability-indicating Assay and its impurities method by UV detection at 210 nm. The developed UPLC method was validated as per the International Conference on Harmonization (ICH) guidelines.[18-19]

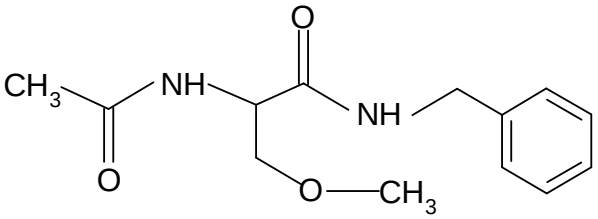

Figure 1.0: Chemical structure of Lacosamide.

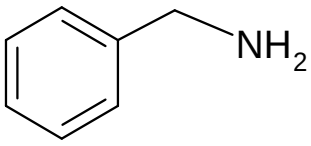

Figure 2: Chemical structure of Benzyl Amine Impurity.

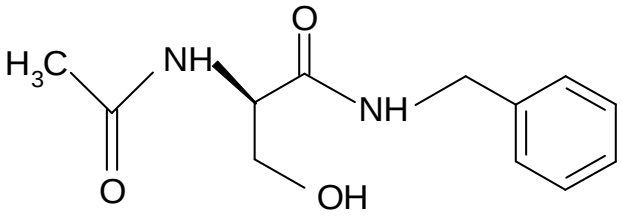

Figure 3: Chemical structure of Hydroxy Impurity.

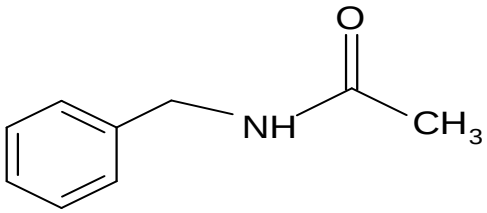

Figure 4: Chemical structure of Acetamide Impurity.

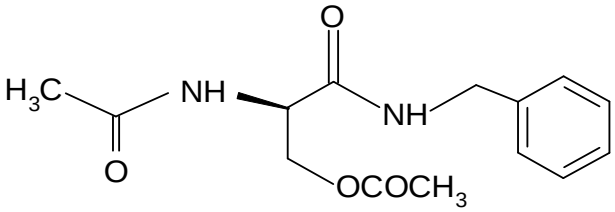

Figure 5: Chemical structure of O-Acetyl Impurity.

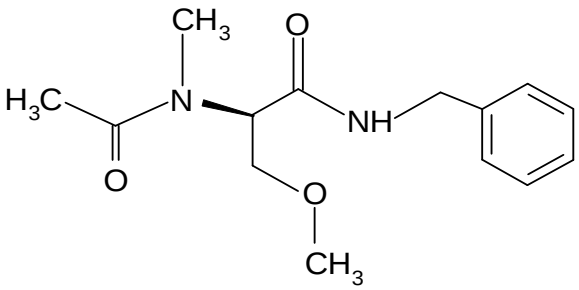

Figure 6: Chemical structure of N-Methyl Impurity.

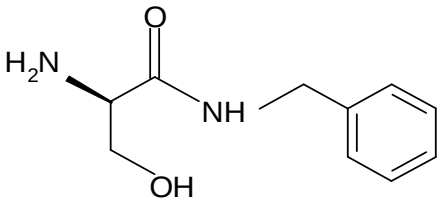

Figure 7: Chemical structure of Hydroxy Amino Impurity.

MATERIALS AND METHODS

Chemicals and Reagents

Lacosamide was synthesized by pharmaceutical chemistry lab of Hebei Medical University (Shijiazhuang, China). High-performance liquid chromatography (HPLC)-grade acetonitrile from J.T. Baker (Phillipsburg, NJ, USA) was used as the mobile phase. Formic acid and ammonium acetate were purchased from Dikma Technologies Incorporation. Analytical grade hydrochloric acid, sodium hydroxide, and 30% H_2O_2 purchased from Tianjin Chemical Reagent Factory (Tianjin, China) were used to degrade lacosamide. Purified water was purchased from Wahaha Corporation (Hangzhou, China).

Analytical Instrumentation

LC–QqLIT-MS analysis was performed using a 3200 QTrap instrument (AB, Sciex, Framingham, MA, USA) coupled to an Agilent (Santa Clara, CA, USA) 1200 HPLC system. The HPLC system consists of an auto-sampler, a quaternary solvent delivery system and a column compartment. A hybrid triple quadrupole linear ion trap mass spectrometer equipped with Turbo V sources was used for detection. Analyst software (version 1.5.2, AB Sciex) was employed for instrument control and data processing.

pH 2.0 Buffer preparation

Accurately weighed 1.36 gm of potassium dihydrogen phosphate in 1000ml of water and pH 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.

Preparation of standard stock 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.

Preparation of diluted standard solution

Pipetted out 5ml of above solution and transferred into 100ml volumetric flask and adjusted the volume with diluent and mixed well. Further transferred 4ml of above solution into 100ml volumetric flask and adjusted the volume with diluent and mixed well.

Preparation of system suitability solution

Pipetted out 5ml of above standard stock solution and transferred into 100ml volumetric flask and adjusted the volume with diluent and mixed well. Further transfered 4ml of above solution into 100ml volumetric flask and adjusted the volume with diluent and mixed well.

Preparation of placebo solution

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

Preparation of test solution

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

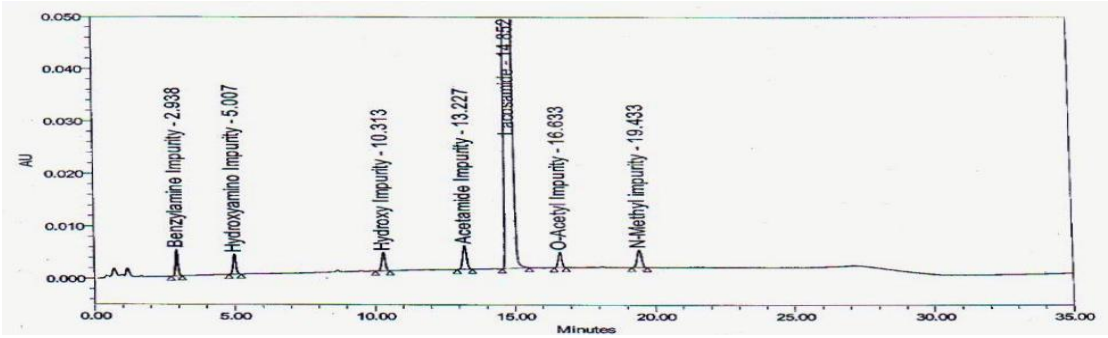

Figure 8: Chemical structure Chromatogram for Reference solution by optimized method.

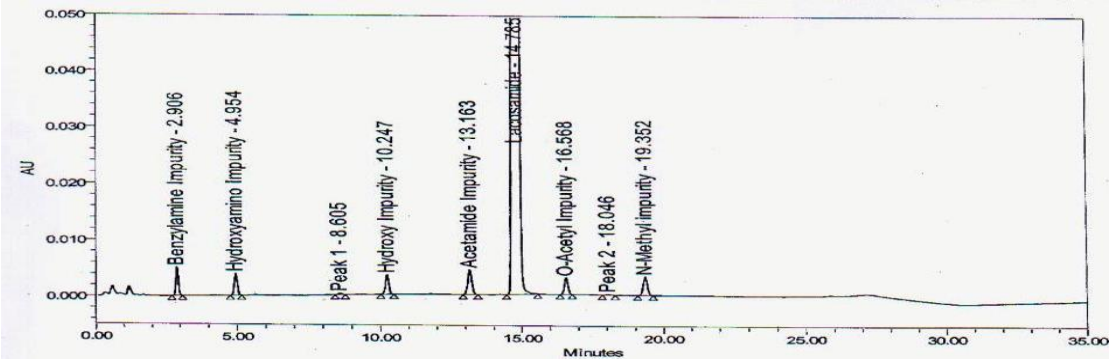

Figure 9: Chemical structure Chromatogram for sample solution by optimized method Optimized Chromatographic Conditions.

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)
Sonication time : More than 10 min to degas mobile phase
Mode : Gradient
Flow Rate : 1.5 mL/min
Column Temperature : 45^0C
Injection Volume : 10 µL
Wave Length : 210 nm
Run time : 35 minutes
Detector : UV-detector

Table No. 1: RRT'S of impurities.

S. No	Impurity Name	RRT
1	Benzyl Amine Impurity	0.19
2	Hydroxy Amino Impurity	0.33
3	Hydroxy Impurity	0.69
4	Acetamide Impurity	0.89
5	Lacosamide	1.00
6	O-Acetyl Impurity	1.12
7	N-Methyl Impurity	1.31

System Suitability

The system suitability studies were carried out as specified in USP. System suitability testing is an integral part of any analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system factor are parameters that are normally used in assessing the column performance. These parameters include column efficiency, resolution, tailing factor, related standard deviation, number of theoretical plates, relative retention time and capacity factor.

PRECISION

Precision of the test method by injecting six test samples prepared by spiking all the impurities at specification limit to the target concentration and also precision study for diluted standard, by spiking working standard at specification level (maximum allowable % level for unknown impurity) on placebo. Injected the solutions in the chromatographic system as per

the method, then calculated the % of impurity of individual % Relative standard deviation of diluted standard for all the impurities. Tabulated the results in the table given below.

Table No. 2: Results for Repeatability of Impurities.

S.no	% Benzyl amine Impurity	% Hydroxy amino Impurity	% Hydroxy Impurity	% Acetamide impurity	% O - Acetyl Impurity	% N - methyl Impurity
1	0.21	0.19	0.19	0.22	0.19	0.18
2	0.22	0.21	0.21	0.21	0.19	0.19
3	0.19	0.19	0.22	0.23	0.21	0.20
4	0.19	0.19	0.21	0.24	0.19	0.17
5	0.21	0.21	0.22	0.20	0.21	0.20
6	0.22	0.20	0.19	0.22	0.20	0.21
Avg	0.21	0.20	0.21	0.22	0.20	0.19
% RSD	0.17	0.19	0.27	0.20	0.16	0.24

ACCURACY

To determine the accuracy of the test method samples were prepared by homogeneous blend of tablet as per manufacturing formula or use the drug product, API and placebo can also be individually weighed and mix for each solution. Prepare stock solutions of all known impurities prepare sample solutions in triplicate (6-preperations for higher level) by spiking test preparation with impurity stock solutions at 50%, 100% and 150% of specification limit to the target concentration. Injected the solutions in the chromatographic system as per the method then Calculate the % recovery of all the impurities. Tabulated the results in the table given below and accuracy at 150% level should be used in range. Accuracy determination is done for every impurity while doing related substances validation to determine the amount of impurity found.

LIMIT OF QUANTIFICATION AND LIMIT OF DETECTION

Injected the solutions in to the HPLC System. If the drug product contains impurities more than LOQ, perform accuracy at LOQ on placebo spiked with API. In signal to noise ratio method, determine the LOD of API and known impurity by identifying the concentration which gives a signal to noise ratio about 3.

Table No. 3: Results of LOD & LOQ.

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	0.009	4659	2.38	0.027	14337	10.21

Impurity						
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

LINEARITY OF DETECTOR RESPONSE

To demonstrate the linearity of detector response for Lacosamide and its impurities prepare not less than six solutions with concentrations ranging from Limit of quantification level to 200 % of the target concentration at specification limit, inject in to the chromatographic system by following concentration in the test method. Injected the solutions in to the chromatographic system and the calibration curve was plotted using peak area ratio Vs concentration of the standard solution. From the calibration curve, the slope and intercept were calculated. Summarized the results in the table given below. 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.

SPECIFICITY

Specificity is to ensure that the signal measured comes from the substance of interest, and that there is no interference from excipient, degradation products and impurities. Prepared the placebo solution in duplicate as per the test procedure. Injected into chromatographic system and checked the interferences due to placebo and blank peaks at the retention time of Lacosamide and known impurities.

RUGGEDNESS

To demonstrate ruggedness of related substances method, Conduct system-to-system variability on two HPLC systems (of the same or different manufacturer) by the same or different analyst qualified during analyst variability and using the different column qualified during the column to column variability. Injected the precision of the test method by injecting test samples prepared by spiking all the impurities at specification limit to the target concentration. And also precision study for diluted standard, by spiking working standard at

specification level (maximum allowable % level for unknown impurity) on placebo. Injected the solutions in the chromatographic system as per the method then calculated the individual % Relative standard deviation of diluted standard and all the impurities.

Table No. 4: Results of solution stability on bench top.

S.No	Impurity	Initial	24 hours	% Difference	48 hours	% Difference
1	Benzyl Amine Impurity	0.203	0.210	0.007	0.209	0.006
2	Hydroxy Amino Impurity	0.204	0.210	0.006	0.212	0.008
3	Hydroxy Impurity	0.217	0.225	0.008	0.225	0.008
4	Acetamide Impurity	0.200	0.206	0.006	0.207	0.007
5	O-Acetyl Impurity	0.228	0.234	0.006	0.236	0.008
6	N-Methyl Impurity	0.203	0.209	0.006	0.211	0.008
7	Total Impurity	1.278	1.318	0.040	1.324	0.046

ROBUSTNESS

It is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and indication of its suitability during normal usage.

Effect of variation in mobile phase composition

The robustness of assay method is demonstrated by changing the composition of organic phase Mobile phase A and Mobile phase B Acetonitrile as 90% and 110% instead of 100%. Standard solutions was prepared as per test procedure and injected 6 replicates into the chromatograph with variation of organic phase composition in mobile phase as 90% and 110% and evaluated the system suitability parameters. The %RSD, tailing factor and theoretical plates of Lacosamide standard was found to be within limits.

Table No. 5: Results of Effect of variation in mobile phase.

S. No	System suitability parameters	Acetonitrile 90%	Acetonitrile 100%	Acetonitrile 110%
1	Tailing Factor for standard preparation	1.0	1.0	1.0
2	Theoretical Plates for Lacosamide Standard solution	49078	48073	47051
3	The %RSD for Lacosamide from Six replicate injections of standard solution	0.9	0.8	3.5

Effect of variation in buffer pH

Robustness of assay method is demonstrated by changing the pH for 1.8 and 2.2 instead of specified pH (2.0). By injecting the 6 replicate injections of standard in 1.8 and 2.2 pH and found that system suitability parameters are passed. The % RSD, tailing factor and theoretical plates of Lacosamide standard are within the limits.

Table No. 6: Results of Effect of variation in Buffer pH.

S. No	System suitability parameters	pH 1.8	pH 2.0	pH 2.2
1	Tailing Factor for standard preparation	1.2	1.0	1.2
2	Theoretical Plates for Lacosamide Standard solution	49184	50350	54090
3	The %RSD for Lacosamide from Six replicate injections of standard solution	2.8	2.3	7.1

Effect of variation in mobile phase Flow Rate

Robustness of assay method is demonstrated by changing the flow rate for 1.3 ml/min and 1.7 ml/min instead of specified flow rate (1.5 ml/min). By injecting the 6 replicate injections of standard in 1.3 ml/min and 1.7 ml/min flow rate and found that system suitability parameters are passed. The % RSD, tailing factor and theoretical plates of Lacosamide standard are within the limits.

Table No. 7: Results of Effect of variation in mobile phase Flow Rate.

S.No	System suitability parameters	Flow 1.3ml/min	Flow 1.5ml/min	Flow 1.7ml/min
1	Tailing Factor for standard preparation	1.0	1.1	1.1
2	Theoretical Plates for Lacosamide Standard solution	49869	52205	46239
3	The %RSD for Lacosamide from Six replicate injections of standard solution	6.1	6.1	1.1

Effect of variation in Temperature

Robustness of assay method is demonstrated by changing the temperature 40^{o}C and 50^{o}C instead of specified temperature (45^{o}C). By injecting the 6 replicate injections of standard in 0 temperature 40^{o}C and 50^{o}C and found that system suitability parameters are passed. The % RSD, tailing factor and theoretical plates of Lacosamide standard are within the limits.

Table No. 8: Results of Effect of variation in Temperature.

S.No	System suitability parameters	40 ^{o}C	45^{o}C	50^{o}C
1	Tailing Factor for standard preparation	1.0	1.0	1.0
2	Theoretical Plates for Lacosamide Standard solution	49893	48640	47110

3	The %RSD for Lacosamide from Six replicate injections of standard solution	0.4	1.0	1.0

SUMMARY AND CONCLUSION

The scope and objective of the present work is to optimize the liquid chromatography conditions to separate the drug from its degradation products on a reverse phase HPLC, C18 column and to establish a validated stability-indicating Assay and its impurities method by UV detection at 210 nm. The developed HPLC method was validated as per the USP, International Conference on Harmonization (ICH) guidelines. The literature survey reveals that there is available stability indicating RP-UPLC, RP-HPLC, HPTLC, UV Spectroscopic methods for assay of Lacosamide in tablet formulation. Furthermore, to the best of our knowledge; no stability-indicating HPLC method is reported in the literature for related substances of Lacosamide in injection formulation. In this method 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.

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. At the optimum conditions mentioned above. The total run time required was 35mins. The linearity and range was established over the range of LOQ – 200 % concentration range for Lacosamide and their related substances. The correlation coefficient of Lacosamide and their related substances was found to be 0.999.

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.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Azidus Laboratories, Chennai, India, for their valuable support in providing a literature survey and facilitating this research.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest related to this research.

REFERENCES

1. Sanjay B. Bari, Bharati R. Kadam, Yogini S. Jaiswal, Atul A. Shirkhedkar, Impurity profile: Significance in Active Pharmaceutical Ingredient, Eurasian Journal of Analytical Chemistry Volume 2, Number 1, 2007.
2. Parmar MD1*, Nimavat KS2, Vyas KB3, Rao DVNS1, Pande R1. A Stability-Indicating Liquid Chromatographic Method for the Quantification of New Anti-Epileptic Drug Lacosamide and its Intermediates. (IJPRS). 2012. research article v-1. issn no: 2277-7873 pg: 40-47.
3. Srihari Molleti1, Vinay Rao2 and K. N. Jayaveera3. Stability indicating RP-UPLC method for the determination of Lacosamide and its impurities in Bulk drugs and its pharmaceutical dosage form. Der Pharma Chemica. 2013. ISSN 0975-413X. 5(1):81-89.
4. Saroj Kumar Raul1*, Anjan Kumar Mahapatra, Ravi Kumar B. V. V. and Ajaya Kumar Patnaik. Stability indicating RP-HPLC method for the estimation of Lacosamide in Bulk and pharmaceutical dosage form.JCPR, 2013; ISSN: 0975-7384, 5(11): 732-739.
5. Kalyan Chakravarthy. V* and Gowri Sankar. D. Stability indicating Hplc method for determination of Lacosamide and its degradants/impurities in bulk and pharmaceutical formulation. rasayan j.chem., 2012; 5(3): 293-310, issn: 0974-1496
6. Apexa patel1, Suhagia. b.n 2,Aarpit patwari. stability indicating assay method for quantification of Lacosamide in bulk and its pharmaceutical dosage form and characterization of major degradation products. IJPPS, 6(1): ISSN- 0975-1491.
7. Vudagandla Sreenivasulu, Dokku Raghava Rao, Uma Maheswari BN, Samar K Das, Abburi Krishnaiah, Development and validation of a stability-indicating RP – HPLC method for determination of Lacosamide. JPBCS, 2011; 2(4): Page No. 1
8. Usmangani.K, Chhalotiya, Kashyap.K, Bhatti, Dimal.A. Shah, Sunil.L, Baldania, Jigar.R.Patel, stability-indicating liquid chromatographic method for quantification of new anti-epileptic drug lacosamide in bulk and pharmaceutical formulation. Chemical Industry & Chemical Engineering Quarterly, 2012; 18(1): 35−42.

9. Sachin Shah, S. G. Vasantharaju*, Arumugam Karthik and Muddukrishna B.S. Development and validation of stability-indicating assay method for lacosamide by RP-HPLC, Elixir Pharmacy, 2011; 38: ISSN: 2229-712X, Pg No. 4174-4177.
10. A. B. N. Nageswara Rao, G. Rohini Reddy, Sunil Kumar Chaitanya, MD. Abdul Shoeb, Akram Khan, MD. Azeem Hussain, UV-Visible spectrophotometric method for the determination of Lacosamide in its pure and tablet dosage form, Der Pharmacia Lettre, 2012; 4(6): 1737-1741.
11. Ganji Ramanaiah*, D. Ramachandran, G.Srinivas, Srilakshmi, Purnachanda Rao. Development and validation of UV spectroscopy method for estimation of Lacosamide in bulk and formulations. Research Drops PharmaInterScience Publishers, Int J Pharm Biomed Sci., 2012; 3(1): 10-12. ISSN No: 0976-5263
12. K.Sai Sumanth*1, C. Jose Gnana Babu1, R. VenkataMahesh1, S. Muneer1, M. Balaji1. Development And Validation Of Rp-Hplc Method For Estimation Of Lacosamide In Bulk And Its Pharmaceutical Dosage Form. International Journal of Pharmaceutical Research & Analysis, ISSN: 2249 – 779X, Vol 2 / Issue 1/ 2012/ 1-5.
13. United Nations International Drug Control Programme Vienna. Drug Characterization/ Impurity Profiling, United Nations Publication Sales No. E.01.XI.10 ISBN: 92-1-148139-2.
14. European Medicines Agency, Evaluation of Medicines for Human Use. Doc.Ref.: EMEA/460925/2008. Assessment Report For Vimpat International Nonproprietary Name: Lacosamide, Procedure No. EMEA/H/C/000863.
15. Sanjay B. Bari, Bharati R. Kadam, Yogini S. Jaiswal, Atul A. Shirkhedkar. Impurity profile: Significance in Active Pharmaceutical Ingredient. Eurasian Journal of Analytical Chemistry Volume 2, Number 1, 2007.
16. European Pharmacopoeia 5.0, 5.10. Control Of Impurities In Substances For Pharmaceutical Use. 01/2005:51000.