Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 1844-1850 | ISSN: 2251-6719

A Quantitative Reliable RP-HPLC Method Development and Validation for the Monitoring of Acetic Acid in Lacosamide Anti Epilepsy Drug

Vavilala Vishweshwar[1], Suman Pallapati[2], B. B. V. Sailaja[3*], Korupolu Nagu[4]

[1]Research Scholar, Department of Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India 530003 & Dr. Reddy's Labs, Hyderabad, Telangana, India.
[2]Research Scholar, Department of Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India – 530003.
[3]Professor, Department of Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India – 530003.
[4]Research Scholar, Department of Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India.530003.
[*]Corresponding Author: B.B.V.Sailaja, Professor, Department of Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India – 530003.

(Received: 05 October 2024 Revised: 15 November 2024 Accepted: 21 December 2024)

KEYWORDS*:* Lacosamide, Acetic Acid, Anti-Epilepsy Drug, and Epilepsy.

ABSTRACT:
Lacosamide is prescribed as a partial-onset seizure medication and as an adjuvant for the treatment of primary generalized tonic-clonic seizures. A validated reverse phase HPLC method has been developed and validated to determine the acetic acid content of Lacosamide in a simple, precise, affordable, and accurate manner. There is a daily intake limit for acetic acid, which is classified as a Class III solvent under ICH recommendations. By monitoring of acetic acid in Lacosamide using this method, the final chromatographic conditions were obtained using a Kromasil-C18 (250 x 4.6mm, 5µm) and a mobile phase that was prepared by orthophosphoric acid buffer (1.0 ml of orthophosphoric acid dissolved in 1 liter of HPLC grade water) and acetonitrile. At a flow rate of 1.0 mL/min and a detection wavelength of 210 nm, a tiny runtime was achieved within 30.0 minutes. With a linear regression curve (R^2 = 0.99), the linearity was demonstrated in the range of 0.025–7.5mg/mL of working concentration, with limits of detection (LOD) and quantitation (LOQ) of 8.2ppm and 24.9ppm, respectively. The suggested approach was simple, sensitive, and capable of directly analyzing the amount of acetic acid without the need for a reaction.

1. Introduction:

People of all ages are affected by epilepsy, a chronic, non-communicable brain disease. One of the most prevalent neurological disorders around the world, epilepsy affects about 50 million people worldwide. The mainstay of treating epilepsy since 1989 has been prescription antiepileptic medication. Nevertheless, the prognosis of intractable epilepsy has not been much improved by current antiepileptic medications, and more are required. Antiepileptic medications that act on novel therapeutic targets with improved efficacy and a smaller amount side effects are specifically needed. Most adult patients who have partial-onset seizures are treated with Lacosamide (Figure1), a third-generation antiepileptic medication, as an adjuvant. Lacosamide may interact with collapses in response mediator protein-2 and preferentially increase the delayed inactivation of voltage-gated sodium channels. The exact efficacy of these unique mechanisms of action in treating epileptic patients will only become evident as more patients are taken the drug [1-7].

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1844-1850 | ISSN: 2251-6719

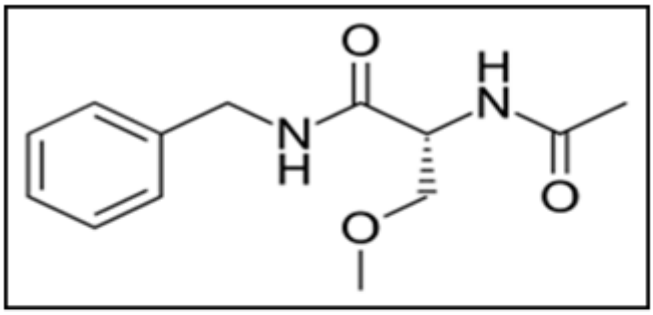

Figure 1: Structure of Lacosamide

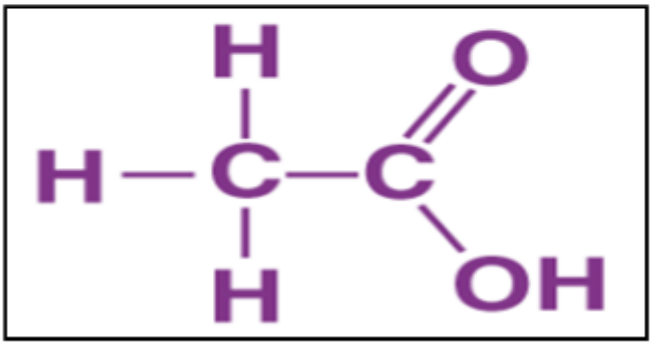

Figure2: Structure of Acetic Acid

The USFDA has mandated that any residual solvents found in pharmaceutical products and components be profiled as of July 1, 2008. The USFDA recommends thorough profiling of residual solvents before approving them for filing, as they were officially recognized in USP under General Chapters <467>. Additionally, the FDA advises limiting the amount of residual solvents in pharmaceutical products and substances that aren't covered by a USP monograph. According to ICH recommendations for residual solvents under ICHQ3C (R4), acetic acid (Figure2) is categorized as a class III residual solvent and has a daily intake limit of 50 mg. Any new substance found in a medication component should always be considered as an impurity, even if it is innocuous [8-10]. Acetic acid thus is deemed to be an impurity. The residual acetic acid level of all drug substance and their formulations must be monitored in all pharmaceutical production units. Hence, its presence needs to be monitored. A survey of the literature indicates that several liquid chromatographic techniques [11–18] have been documented for the quantification of Lacosamide in tablet and bulk dose forms. However, only a small number of liquid chromatography methods [19–23] have been published for determining the amount of acetic acid in various pharmaceutical compounds. But none of them are especially dedicated to studying the acetic acid in Lacosamide. Therefore, an easy technique is usually needed for the assessment of acetic acid in Lacosamide with acceptable analytical time. Establishing chromatographic conditions that could be utilized for determining acetic acid in Lacosamide is the aim of this endeavor.

2. Materials and Methods:

2.1. Chemicals, Reagents and Samples:

"Qualigens fine chemicals Ltd, India" supplied acetonitrile, and Sun Pharma, Mumbai, provided Lacosamide. The acetic acid in Lacosamide's active medicinal components was determined using materials from "Standard, India" (methanol and dichloromethane, HPLC kind), "Rankem, India" (orthophopshoric acid), "Milli-Q system, USA" (water), and "Merk, India" (acetic acid).

2.2. Instrument and Software:

The Waters 'HPLC', a system with a UV- detector, was used to do the data acquisition. Empower Software was used for the method screening and optimization.

Preparation of Buffer: 1.0ml of Orthophosphoric acid was diluted in 1 liter of HPLC grade water and filter through 0.45µ filter paper.

Mobile Phase (MP) preparation: Mobile Phase A: buffer and Mobile Phase B: acetonitrile Both are filtered through 0.45µm membrane filter paper.

Diluent preparation: Use Mobile Phase as a diluent

Preparation of acetic acid standard solution:

In a 100 mL clean, dried volumetric flask (VF), accurately weighed, and later transferred 25mg of acetic acid as the working standard. Add diluent and sonicated

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1844-1850 | ISSN: 2251-6719

to completely dissolve it, then add more diluent to make the volume up to the limit with the same diluent. Taken up 5.0 mL of the aforementioned stock solution into a 50 mL VF and added the diluent until the desired concentration is achieved.

Preparation of Lacosamide standard solution:

Accurately weighed and transfer 33.5mg of standard into a 100 mL clean dried VF added the diluent and sonicated it up to 30 minutes to dissolve, and centrifuge for 30 minutes to dissolved it completely and make volume up to the mark with the above mentioned solvent. Then it is filtered through 0.45 micron filter. Further pipette out 3 mL of the directly above stock solutions into 50 mL of VF and dilute up to the mark with diluents.

Preparation of Sample (Lacosamide) Solution:

Accurately weighed and transfer 1.0g of Lacosamide sample into a 50 mL clean dried VF added the diluent and sonicated it up to 30 minutes to dissolve, and centrifuge for 30 minutes to dissolved it completely and make volume up to the mark with the above mentioned solvent. Then it is filtered through 0.45 micron filter. (Con C.: 20 mg/mL).

2.3. Chromatographic system:

Analysis was accomplished on an HPLC system, containing of a binary solvent manager, sample manager and a UV detector. The analytical column was a Kromasil-C18 (250 x 4.6mm, 5µm). The separation of acetic acid and Lacosamide were achieved by gradient elution using OPA- buffer and CH_3CN. These were the final conditions that were chosen as optimized conditions.

Validation of the proposed method:

Specificity:

The specificity of developed HPLC method was demonstrated by analyzing the diluent, test sample, test sample spiked with acetic acid at their specification level individually. Their spectral peak purity data was recorded.

Linearity:

The linearity of acetic acid was demonstrated between 25% and 150% of the reference concentration by using at least six calibration levels (25, 50, 75, 100, 125, and 15%). We used linear regression to evaluate the linearity. The matching concentrations and the peak areas of standard solutions were plotted. An equation describing linearity was utilized to calculate the correlation coefficient.

Accuracy:

The recovery experiments were conducted to determine the accuracy of the method for the quantification of acetic acid in Lacosamide. The study was carried out in triplicate at four different concentration levels viz. 50, 100 and 150% of the specification level. Percentage recoveries were calculated.

Precision:

For method precision, six individual measurements of Lacosamide sample spiked with acetic acid were analyzed, calculated the % of acetic acid from each measurement and determined their mean, standard deviation and relative standard deviation. An intermediate precision experiment performed by different analyst, column and instrument in the same laboratory. Obtained results of method precision and intermediate precision were compared.

Sensitivity:

The ICH guideline's signal-to-noise method was employed to evaluate the acetic acid's limit of quantitation (LOQ) and limit of detection (LOD). After gradually diluting acetic acid solutions were injected into the RP-HPLC, the signal-to-noise (S/N) ratio was determined at each concentration.

Robustness:

Robustness of the method was evaluated by making deliberate changes in flow rate (± 10%), mobile phase organic composition (± 5% absolute). At each condition determined the resolution between acetic acid and Lacosamide was determined. All the other mobile phase components were held constant as described above.

3. Results and Discussion:

3.1. Method development and optimization:

The major criteria required for the development of the effective RP-HPLC method for the assessment of acetic acid in Lacosamide. The method should be accurate, precise, reproducible, and robust, so that it can be used on a regular basis in QC-labs. To achieve this, the following trials were conducted, and ultimately the chromatographic conditions were optimized. The retention of Lacosamide increased with an increase in column length, so a column Kromasil-C18 (250 x 4.6mm, 5µm) was selected to provide the possible run time without compromising to increase symmetry shape of the drug. The retention time of the two components was significantly impacted by a change in the percentage of ACN in the mobile phase. The final gradient run was chosen with regards to the peak shape, analysis time and tailing. Regarding backpressure in addition to run time, the flow rate of 1.0 mL/min was carefully chosen as the most effective approach. The standard and sample concentration of acetic acid 20 mg/mL and injection volume of 20 µL were also optimized with regard to peak response, resolution and tailing. Lacosamide standard and sample concentrations responded satisfactorily at 210 nm, making it possible to determine their acetic acid content results in a single run. Acetic acid and Lacosamide were eluted in reasonable time of approximately 5.0 minutes and 12.5 minutes. Typical

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1844-1850 | ISSN: 2251-6719

chromatograms of the blank, standard and sample were shown in Figure 3, 4 and 5 respectively.
The final optimized chromatographic conditions were achieved with a Kromasil-C18 (250 x 4.6mm, 5µm), and a mobile phase was adapting by gradient elution at ambient column temperature. Lacosamide was detected (PDA-detector) at 210 nm, and a satisfactory separation was achieved in 5.0 minutes of runtime with a flow rate of 1.0 mL/min., in Waters High Performance Liquid Chromatography'.

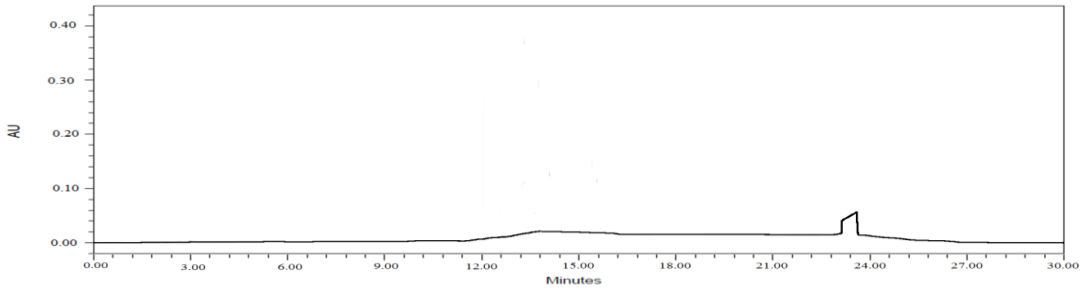

Figure 3: Blank chromatogram of Lacosamide

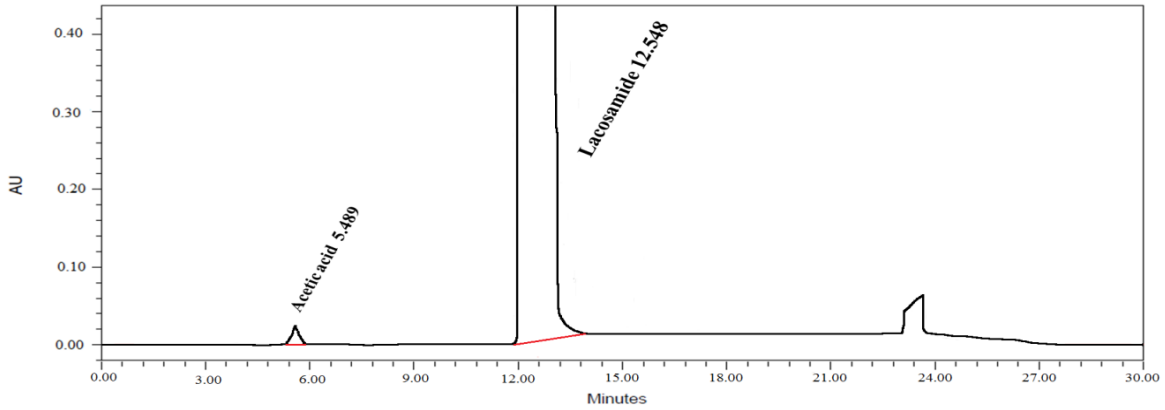

Figure 4: Standard chromatogram of Lacosamide

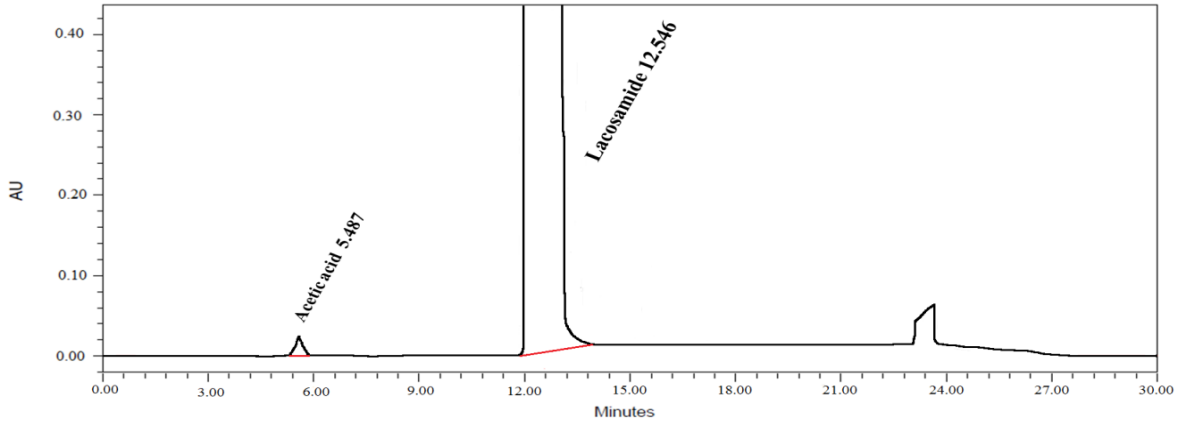

Figure 5: Sample chromatogram of Lacosamide

The proposed developed method suggested that no interference from the degradation products, making it superior method.

3.2. Analytical method parameters and its validation:
After a successful development phase, the method was put through method validation in accordance with ICH principles. The proposed RP-UPLC method was validated using the approved procedure to assess acceptable validation properties and show that it is appropriate for its intended use.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1844-1850 | ISSN: 2251-6719

Table 1: Summary of validation parameters for monitoring of acetic acid in Lacosamide

S. No	Parameters		Acetic acid
1	Specificity		No interferences
2	Linearity range		25-7475 µg/ml
3	Regression line equation		Y=14.09x -161.78
4	Correlation co-efficient		0.99996
6	Precision	Repeatability	0.87
7	(% RSD)	Inter-day precision	0.81
8	LOD		8.2 µg/ml
9	LOQ		24.9 µg/ml
10	Accuracy(Recovery)		98.5-101.2%

Specificity:
By comparing blank, Lacosamide, and acetic acid impurity at various relative retention times (RRTs), the method's specificity was demonstrated. According to the experimental data [Table 1], the chromatogram [Figures 3-5] of Lacosamide and acetic acid does not contain any interfering peaks at the retention time. Acetic acid and Lacosamide are clearly separated in the modified approach, making it selective for determining the amount of acetic acid present.

Linearity:
The acceptability of the method for the quantitative determination of acetic acid was confirmed by the linearity regression studies. To determine the linearity range, the obtained data was evaluated using linear regression analysis, obtaining the correlation coefficient, slope, and intercept. For acetic acid, this method exhibits a linear range of 25µg/ml to 7475 µg/ml (i.e., LOQ% to 150%) with a correlation coefficient larger than 0.999 [Figure 6].

Table 2: Linearity data

Linearity Level	Lacosamide				
	Concentration (µg/mL)	Peak's Area	Slope	Intercept	CC
LOQ	25	394	14.09	161.78	0.99996
50%	2492	34659			
75%	3737	51845			
100%	4983	70401			
125%	6229	87816			
150%	7475	105172			

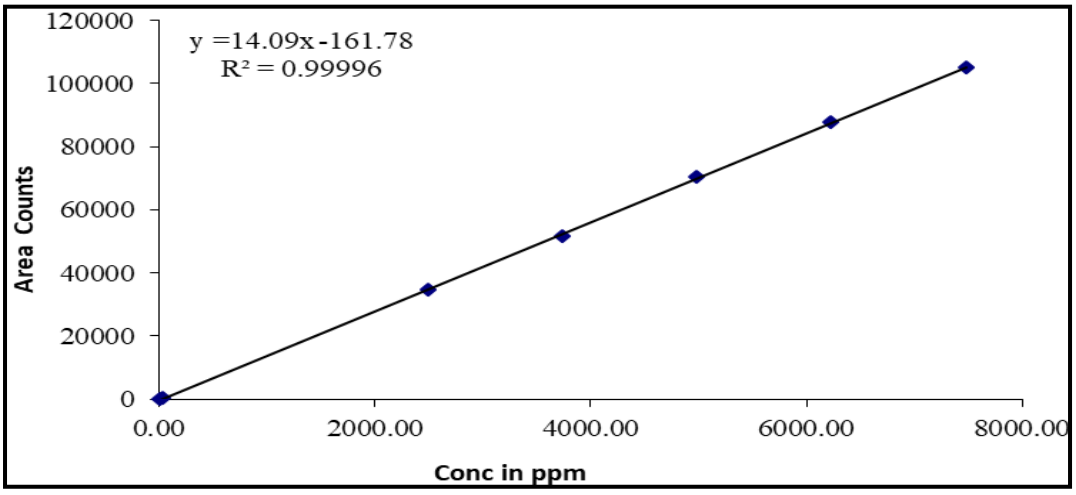

Figure 6: Linear graph for acetic acid in Lacosamide

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(6), 1844-1850 | ISSN: 2251-6719

Precision:
Six different measurements of the acetic acid-spiked Lacosamide sample were examined for method precision. The percentage of acetic acid in each measurement was computed, and the mean, standard deviation, and relative standard deviation were ascertained. a moderately accurate experiment carried out in the same lab using a different analyst, column, and equipment. The method precision and intermediate precision results obtained were compared.

Accuracy:
The purpose of the recovery studies was to evaluate the precision of the acetic acid quantification method in Lacosamide. At three distinct concentrations levels—50, 100, and 150% of the specification level the investigation was conducted in triplicate. Recoveries as a percentage were computed.

LOD and LOQ:
Many methods for determining these two parameters are described in ICH Guideline Q2 (R1). They comprise visual inspection, signal-to-noise ratio, calibration curve's- slope and standard deviation of response. The S/N ratio technique was used for the current study. According to ICH recommendations, the LOD was confirmed to be a concentration (in µg/mL) with a S/N ratio of at least 3, and the LOQ was corroborated to be a concentration (in µg/mL) with a S/N ratio of at least 10 for acetic acid LOD and LOQ results are 8.2 and 24.9 respectively, which are represented in Table 1.

Robustness:
Resolution, tailing, and theoretical plates of acetic acid did not significantly alter when minor deliberate modifications, such as increasing and decreasing the MP's flow rate by 10% and column temperature by 10%, were made to the chromatographic conditions. The results and standard method system suitability parameters are shown in Table 3. As a result, the method's robustness to variations in the before demonstrated conditions has been shown.

Table 3: Robustness of the proposed method

Parameters	**Change in Flow (1.0 mL/min)**		**Column temperature (30°C)**	
Variations	0.9 mL/min	1.1 mL/min	**27°C**	**33°C**
Average area of Acetic acid	396	395	393	394
SD of Area	1.47	3.06	2.93	3.31
% RSD of Acetic acid	0.37	0.78	0.75	0.84

4. Conclusions:

In conclusion, a gradient RP-HPLC method for quantification of acetic acid present in Lacosamide was developed also the validation data demonstrates a high precision and accuracy, which proves the reliability of the developed method. The developed method was validated to ensure the compliance in accordance with the regulatory guidelines. The method shows the best baseline separation, good resolution, symmetric peaks, and a wider concentration range of 0.024–7.475 mg/mL for acetic acid, as well as complying with the system's suitable conditions. Further, the observed experimental values of LOD and LOQ for acetic acid were found to be 8.2µg/mL and 24.9µg/mL, respectively. The method was found to be sensitive, accurate, precise, robust and useful for its intended use. In addition to the above the short runtime and cheaper mobile phase involved in the method are advantageous for analyzing routine quality control samples for the quantification of acetic acid in Lacosamide bulk drug.

Acknowledgement:
The author would like to thank Professor B. B. V. Sailaja, Professor for accomplished my research work

Conflict of interest: The authors declare that there are no conflicts of interest.

References:

1. Kestelyn C, Lastelle M, Higuet N, Dell'Aiera S, Staelens L, Boulanger P, et al. A simple HPLC-UV method for the determination of lacosamide in human plasma. Bioanalysis.2011; 3(22): 2515–2522.
2. Shah S, Vasantharaju SG, Arumugam K, Muddukrishna BS, Desai N. Development of a sensitive bioanalytical method for the quantification of lacosamide in rat plasma. Application to preclinical pharmacokinetics studies in rats. Arzneimittel-Forschung. 2012; 62(5): 243–246.
3. Kim SJ, Koo TS, Ha DJ, Baek M, Lee SK, Shin DS, et al. Liquid chromatography-tandem mass spectrometry for quantification of lacosamide, an

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 1844-1850 | ISSN: 2251-6719

antiepileptic drug, in rat plasma and its application to pharmacokinetic study. Biomedical chromatography: BMC. 2012; 26(3): 371–376.

4. Chen J, Shen Y, Xia H, Chen X, Xu RA, Lin G, Dai G. Development of a UPLC-MS/MS method for the determination of lacosamide and its metabolite and its application to drug-drug interaction. Frontiers in pharmacology. 2023; 14: 1265252.
5. Furugen A, Nishimura A, Umazume T, Ishikawa H, Narumi K, Kobayashi M. Simple and validated method to quantify lacosamide in human breast milk and plasma using UPLC/MS/MS and its application to estimate drug transfer into breast milk. Journal of pharmaceutical health care and sciences.2023; 9(1): 26.
6. Martinez SE, Bowen KA, Remsberg CM, Takemoto JK, Wright HM, Chen-Allen, et al.High-performance liquid chromatographic analysis of lacosamide in canine serum using ultraviolet detection: application to pre-clinical pharmacokinetics in dogs. Biomedical chromatography: BMC.2012; 26(5): 606–609.
7. Taylor Battellino, Kosuke Ogata, Victor Spicer, Yasushi Ishihama, Oleg Krokhin. Acetic Acid Ion Pairing Additive for Reversed-Phase HPLC Improves Detection Sensitivity in Bottom-up Proteomics Compared to Formic Acid. Journal of Proteome Research. 2023; 22 (1): 272-278.
8. Yoneda N, Kusano S, Yasui M, Pujado P, Wilcher S."Recent advances in processes and catalysts for the production of acetic acid". Applied Catalysis A: General. 2001; 221 (1–2): 253–265.
9. Yamamoto S, Iguchi Y, Shibata N, Takesue M, Tsunoda T, Sato K (April 1998). "[Percutaneous ethanol and acetic acid injection for liver metastasis from colon cancer--two case reports]". Gan to Kagaku Ryoho. Cancer & Chemotherapy.1998; 25 (5): 751–755.
10. Noorbasha K, Shaik A. Determination of residual solvents in paclitaxel by headspace gas chromatography. Futur J Pharm Sci. 2021; 7: 40.
11. Kestelyn C, Lastelle M, Higuet N, Dell Aiera S, Staelens L, Boulanger P, et al. A simple HPLC–UV method for the determination of Lacosamide in human plasma. Bioanalysis. 2011 Nov; 3(22):2515-22.
12. Chakravarthy VK, Shankar DG. HPLC method for determination of Lacosamide S (−) enantiomer in bulk and pharmaceutical formulation. Rasayan Journal of Chemistry. 2011; 4: 744-52.
13. Chakravarthy VK, Sankar DG. Development and validation of RP-HPLC method for estimation of lacosamide in bulk and its pharmaceutical formulation. Rasayan J Chem. 2011; 4(3): 666-72.
14. Parmar MD, Nimavat KS, Vyas KB, Rao DVNS, Pande R, A Stability Indicating Liquid Chromatographic Method for the Quantification of New Anti-Epileptic Drug Lacosamide and its Intermediates, International Journal for Pharmaceutical Research Scholars. 2012; 1: I-3.
15. Sachin Shah, SGVasantharaju, Arumugam Karthik, Muddukrishna BS, Development and validation of stability-indicating assay method for Lacosamide by RP- HPLC, Elixir International Journal. 2011; 38: 4174- 4177.
16. Sai Sumanth K, Jose Gnana Babu C, Venkata Mahesh R, Muneer S and Balaji M. Development and validation of rp-hplc method for estimation of lacosamide in bulk and its pharmaceutical dosage form, International Journal of Pharmaceutical Research and Analysis. 2012; 2(1):1-5.
17. Saroj Kumar Raul. Stability indicating RP-HPLC method for estimation of lacosamide in bulk and pharmaceutical dosage form. J Chem Pharm Res. 2013; 5(11):732- 379.
18. Vudagandla Sreenivasulu, Dokku Raghava Rao, Uma Maheswari BN, Samar K Das and Abburi Krishnaiah. Development and validation of a stability-indicating RP – HPLC method for determination of lacosamide Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011; 2(4):0975-8585.
19. LÜ Hai-hong, LIAO Hai-ming, LI Yong, XU Kang-sen. RP-HPLC Determination of the Content of Acetic Acid in Tyroserleutide. Chinese Journal of Pharmaceutical Analysis, Volume 24, Number 3, 1 May 2004, pp. 286-288(3).
20. Vandana, Alok Kumar Chaudhary. Validated Reverse Phase HPLC Method for the Determination of Acetic Acid Content in Hard Gelatin Capsule Shells from animal origin. Asian J. Research Chem. 3(4): Oct. - Dec. 2010; Page 1058-1061.
21. Rui Xie, Maobing Tu, Yonnie Wu, Sushil Adhikari. Improvement in HPLC separation of acetic acid and levulinic acid in the profiling of biomass hydrolysate.Bioresource Technology. Volume 102, Issue 7, 2011, Pages 4938-4942,
22. Ajay singh rawat, kamlesh chauhan, yogendrasinh parmar, pooja sannigrahi, divyesh patel, chandrakant belwal and anand vardhan.Quantitative Determination of Acetic Acid in Gefitinib by Reverse Phase HPLC. Chem Sci Trans., 2014, 3(3), pp 983-988.
23. Syed Anwar, Pathan Mohd Arif Ali Khan. HPLC based procedure development for monitoring acetic acid in daclatasvir drug. J Adv Sci Res, 2022; 13(1): 333-339.