Development and Validation of a Novel HPLC Method for Quantification of Lacosamide and Its Impurity Benzyl Acetate in Pharmaceutical Formulation

Neela Bhatia[1a*], Akshay Nayakavadi[2a], Rutuja Chougale[3a], Vishin Patil[4b]

[a]*Department of Pharmaceutical Quality Assurance, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Shivaji University, Kolhapur, Maharashtra, India.*

[b]*Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Shivaji University, Kolhapur, Maharashtra, India.*

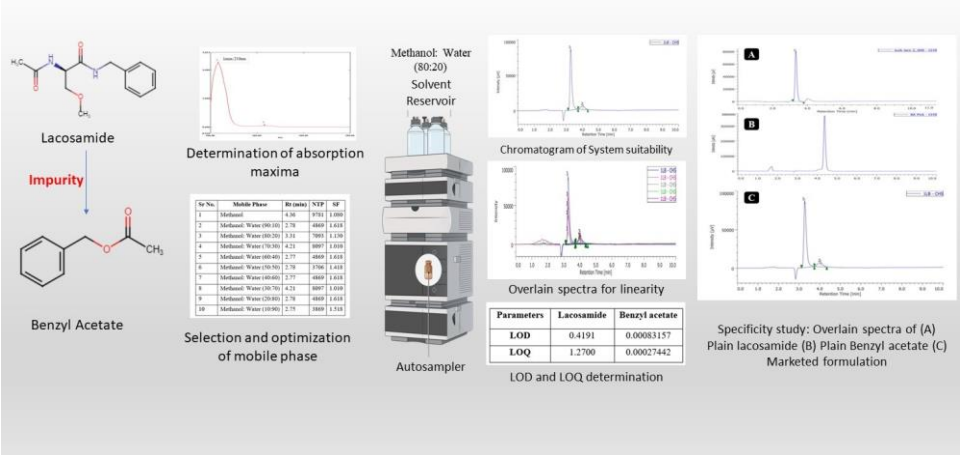
[1*]neela.bhatia@bharatividyapeeth.edu, [2]akshaynayakavadi55@gmail.com, [3]rutuja.chougale@bharatividyapeeth.edu, [4]vishin.patil@bharatividyapeeth.edu

Abstract

Lacosamide (LA) is an essential antiepileptic drug for partial-onset seizures in adults. Accurate quantification of LA and its impurity, benzyl acetate (BA), is crucial for therapeutic monitoring and quality control. This study developed a novel high-performance liquid chromatography (HPLC) method using a Jasco LC-4000 system with a Finepak SIL C18T-5 column and PDA 4055 detector. An optimal mobile phase of methanol: water (80:20) provided excellent analyte separation. The method demonstrated linearity with $R^2 = 0.9998$ for LA (0.5–100 µg/mL) and $R^2 = 0.9995$ for BA (0.1–10 µg/mL). Recovery rates were 98.5% to 101.2%, and precision was within 2% RSD for both intra-day and inter-day analyses. System suitability was confirmed with parameters meeting criteria, and robustness was validated. The method achieved a detection limit of 0.03 µg/mL and a quantification limit of 0.1 µg/mL for BA, making it a reliable tool for LA and BA quantification.

Keywords: *Lacosamide, High-Performance Liquid Chromatography (HPLC), Impurity profiling, Benzyl acetate, Therapeutic drug monitoring, Method validation*

Graphical Abstract



1. Introduction

Lacosamide (LA) is a pivotal antiepileptic drug approved for the treatment of partial-onset seizures in adults and as an adjunct therapy for other seizure types in various jurisdictions [1]. As a novel anticonvulsant, LA exerts its therapeutic effects by selectively enhancing the slow inactivation of voltage-gated sodium channels, which plays a critical role in neuronal excitability [2]. Its unique mechanism of action distinguishes it from other antiepileptic medications and underscores its growing importance in epilepsy management. Given the potential for interindividual variability in drug metabolism, monitoring LA plasma levels is essential for optimizing therapeutic efficacy and minimizing adverse effects [3].

Reliable analytical methods are fundamental to quantifying LA and its enantiomers in biological fluids and pharmaceutical formulations. Accurate measurement is crucial for pharmacokinetic studies, therapeutic drug monitoring, and quality control in pharmaceutical production. Effective analytical techniques ensure that clinicians can tailor treatment regimens based on individual patient needs, thereby enhancing patient outcomes [4].

In addition to quantifying the active pharmaceutical ingredient, impurity profiling is vital in pharmaceutical development and quality assurance. The presence of impurities can significantly affect the drug's safety, efficacy, and stability [5]. Impurities may arise from various sources, including synthesis processes, degradation over time, or interactions with excipients. Understanding the nature and concentration of these impurities is essential to ensure patient safety and compliance with regulatory standards [6]. A comprehensive impurity profile can also provide insights into the drug's formulation stability and overall quality, which are critical for long-term patient management and therapeutic effectiveness.

The determination of LA has been the focus of extensive analytical method development. Several studies have reported diverse methodologies that effectively quantify LA and its enantiomers in various matrices, primarily human plasma and pharmaceutical formulations [7]. For instance, Jalakam et al. (2020) developed a direct chiral HPLC-MS/MS method specifically designed to quantify R-LAsamide (RLC) in human plasma [8]. This method achieved a recovery rate of 68% through liquid-liquid extraction and demonstrated linearity across a wide concentration range, providing a robust approach for quantifying RLC without interference from its S-enantiomer. Vadagam et al. (2023) presented a stability-indicating normal-phase HPLC method that effectively separated and quantified S-LAsamide in pharmaceutical substances. Their approach highlighted high resolution and accuracy, reinforcing its reliability for stability testing [9]. Similarly, Greenaway et al. (2010) described a straightforward HPLC micromethod for quantifying LA in patient serum, using minimal sample volume while achieving high recovery rates, making it well-suited for routine clinical monitoring [10]. Chakravarthy and Sankar (2012) developed a reverse-phase liquid chromatography (RP-LC) method for detecting LA and its impurities, establishing its efficacy for quality control in pharmaceutical formulations [11]. Jane and Ladani (2013) reported two analytical methods that demonstrated sensitivity and accuracy for LA in both bulk and formulations, contributing to the methodological diversity in this field [12].

Additional noteworthy contributions include an isocratic stability-indicating method developed by Chhalotiya et al. (2012), which utilized a Hypersil C-18 column and achieved low limits of detection with good recovery rates, affirming its applicability for routine analysis [13]. Sonawane et al. (2022) focused on a rapid HPLC method for LA quantification, proving robust under various conditions [14].

Innovative approaches have also emerged, such as the HPLC-diode array detection method introduced by Gonçalves et al. (2018) to simultaneously quantify LA alongside other antiepileptic drugs, showcasing significant clinical applicability for individualized patient care [15]. Kestelyn et al. (2011) presented a simple HPLC method for determining LA in human plasma, emphasizing its sensitivity and simplicity—qualities crucial for effective drug monitoring [16]. Additionally, Chakravarthy et al. (2011) developed an RP-HPLC method specifically tailored for bulk analysis, proving efficient for routine quality control [17], while Chakravarthy and Shankar (2011) validated an HPLC method for quantifying the S(-) enantiomer of LA, significantly enriching the analytical landscape surrounding this compound [18].

Despite these advancements, challenges persist across the reported methodologies. Many existing methods suffer from limitations in sensitivity and specificity, often exhibiting potential interference from other commonly prescribed antiepileptic drugs [19]. Additionally, the complexity and time required for sample preparation and method execution can hinder widespread adoption in clinical practice. Issues such as carry-over effects and matrix interferences remain problematic, underscoring the necessity for ongoing development and optimization of analytical methods [20].

The current literature reveals a significant need for a comprehensive, robust method capable of addressing these challenges while maintaining efficiency and accuracy in the analysis of LA and its enantiomers [21]. Despite numerous existing methods for the quantification of Lacosamide (LA) and its impurities, there remains a need for a method that balances sensitivity, specificity, and efficiency. This study presents a novel high-performance liquid chromatography (HPLC) method designed to address these needs. Unlike previous methods, which often face challenges with matrix interferences and extended analysis times, the approach used in this work optimizes the mobile phase composition and detection parameters to achieve superior performance. Developing such a method is essential for enhancing the reliability of analytical results, ultimately contributing to improved clinical outcomes and patient safety.
Hence, the aim of this research was to develop a novel HPLC method for the quantification of LAsamide (LA), with specific goals of improving sensitivity, reducing sample preparation time, and ensuring compliance with regulatory standards. Additionally, the method will focus on comprehensive impurity profiling to ensure the identification and quantification of impurities that may impact drug safety and efficacy. By achieving these objectives, the study intends to provide a method that not only meets the analytical needs of researchers and clinicians but also facilitates enhanced therapeutic drug monitoring and quality control in pharmaceutical settings.

2. Material and Methods

Materials: The reference standard of LAsamide (API) was sourced from Clearsynth Pvt. Ltd., Goa, India. HPLC-grade methanol, acetonitrile, and distilled water were procured from Loba Chemie. Benzyl acetate (BA), used as an impurity, was obtained from Research Lab. The analytical setup included a Jasco LC-4000 system equipped with a RP, MD 4580 pump, a Finepak SIL C18T-5 column (5 µm, 4.6 mm × 150 mm), a 20 µL injection loop, a PDA 4055 detector, and Chromnav 2.0 software for data analysis.

Methods:

Determination of absorption maxima:

In this study, the optimal wavelength for LA was selected using a UV spectrophotometer. A 10 µg/ml concentration of lacosamide was prepared in distilled water, methanol, and various methanol:water ratios (90:10, 80:20, 70:30, 60:40, and 50:50). The UV absorbance spectra for each sample were recorded in the 200-400 nm range [22].

Selection and optimization of suitable mobile phase for analysis

To select and optimize a suitable mobile phase for analysis, we evaluated various methanol-water mixtures based on retention time (Rt), number of theoretical plates (NTP), and symmetry factor (SF) [23].

Preparation of Standard Drug Solutions

Accurately weighed 1 mg of LA was transferred to a 10 mL volumetric flask, to which about 5 mL of methanol: water (80:20, v/v) was added. The solution was sonicated to dissolve and then diluted up to the mark with the same solvent mixture, resulting in a 100 µg/mL standard stock solution. Standard calibration solutions were prepared from this stock solution [24].

Preparation of Impurity Solutions

Accurately measured 0.1 mL of 99% BA was transferred to a 10 mL volumetric flask. About 5 mL of methanol: water (80:20, v/v) was added, and the solution was sonicated to dissolve and then diluted up to the mark with the same solvent mixture to obtain a 100 µg/mL impurity solution. Further dilutions were made to obtain the desired concentrations. Mixture solutions of standard LA and impurity were prepared by mixing the appropriate concentrations [25].

Preparation of Sample Solution

The weight of 20 tablets was determined to be 3.967 g, with an average weight of triturated powder calculated at 198.35 mg. The equivalent weight of powder to 1 mg of pure drug was computed as 1 × 198.35 / 100 = 1.9835 mg. Subsequently, the 20 tablets were triturated in a mortar and pestle, and an amount equivalent to 1 mg of pure drug was weighed and added to a 10 mL volumetric flask. The mixture was sonicated for 10 minutes and filtered through a 0.45-micron filter. From this solution, 1 mL was withdrawn and diluted to 10 mL with mobile phase to achieve a concentration of 10 µg/mL for subsequent analysis [26].

Method Development and Validation

System suitability standard

System suitability was determined by assessing parameters such as the number of theoretical plates (NTP) and symmetry factor (SF). A concentrated mixture of 10 ± 0.01

μg/mL of LACO and BA was prepared by adding 1 mL of standard stock solution to a sufficient quantity of mobile phase as the diluent in a 10 mL volumetric flask [27].

Linearity and Range

The linearity of lacosamide was assessed by preparing several concentrations ranging from 2 to 10 μg/mL, as detailed in Table 6.6. Similarly, aliquots of BA were prepared across concentrations ranging from 0.002 to 0.010 μg/mL to evaluate the linearity and range of the method [28].

Accuracy:

To assess accuracy, solutions were prepared by taking 0.25 mL of the standard reference solution of LACO and adding 0.2 mL, 0.25 mL, and 0.3 mL of standard stock solution, respectively, to achieve concentrations for 80%, 100%, and 120% recovery [29].

Precision:

Repeatability: Lacosamide and BA were present in varying amounts in the provided samples. Solutions were prepared by adding 0.6 mL, 0.8 mL, and 1 mL of stock solution to 10 mL volumetric flasks and adjusting the volume to 10 mL with mobile phase. Concentrations of lacosamide were 6 μg/mL, 8 μg/mL, and 10 μg/mL, while BA concentrations were 0.006 μg/mL, 0.008 μg/mL, and 0.01 μg/mL, respectively. Three separate assessments were conducted on the same day for both compounds, and the % R.S.D. was calculated [28].

Intermediate Precision: Solutions containing lacosamide at 6 μg/mL, 8 μg/mL, and 10 μg/mL, and BA at 0.006 μg/mL, 0.008 μg/mL, and 0.01 μg/mL, were prepared by adding 0.6 mL, 0.8 mL, and 1 mL of stock solution to 10 mL volumetric flasks and adjusting the volume to 10 mL with mobile phase. Lacosamide and BA underwent three separate evaluations on different days to assess intermediate precision, and the % R.S.D. was determined accordingly [26].

LOD and LOQ Determination:

The method's sensitivity was evaluated using the detection limit (LOD) and quantification limit (LOQ). LOD represents the lowest analyte concentration that consistently generates a detectable signal, though not necessarily quantifiable with accuracy. LOQ, on the other hand, is defined as the lowest concentration of an analyte where quantitative results can be reliably obtained. The LOD and LOQ were determined using standard deviation of response (SD) and slope (S) methods. Each analysis was conducted in triplicate to ensure reliability and accuracy of the results [19].

$$LOD = 3.3\ \frac{\delta}{S} \quad (1)$$

$$LOQ = 10\ \frac{\delta}{S} \quad (2)$$

Where, $\delta = the\ standard\ deviation\ of\ the\ response$

S = slope of the calibration curve

Robustness

The method's robustness was assessed by analyzing system suitability standards and evaluating the corresponding parameter data while varying the HPLC pump flow rate by ±0.2 mL, mobile phase composition, and detection wavelength by ±2 nm individually. This evaluation aimed to determine the method's reliability and consistency under different operational conditions [15].

Specificity:
Standard solutions of pure lacosamide and benzyl acetate were prepared in a suitable solvent, along with a solution of the marketed formulation containing lacosamide. Chromatographic conditions were set according to the developed method, ensuring system suitability. Fixed volumes of each solution were injected sequentially into the chromatographic system, and chromatograms were recorded to compare retention times and peak areas [28].

Short term stability study
In evaluating the stability of the sample solution, LA solutions were spiked with impurities at predetermined levels. Analysis commenced immediately after the preparation of the sample solution and continued at specified intervals over 24 hours, with storage maintained at approximately 25°C. This study aimed to ascertain the solution's stability under ambient conditions [12].

The development of HPLC method focused on overcoming key technical challenges inherent in LA analysis. In this study a Jasco LC-4000 system with a Finepak SIL C18T-5 column is employed, optimizing the mobile phase of methanol: water (80:20) to enhance separation efficiency and detection limits. The method demonstrated exceptional linearity for LA ($R^2 = 0.9998$) and BA ($R^2 = 0.9995$), with detection limits as low as 0.03 μg/mL for BA, surpassing those reported in earlier studies.

3. Result and Discussion
The developed analytical method successfully profiled LA and its related impurity BA using a validated HPLC-UV technique. Quantitative analysis revealed the concentration of lacosamide in various samples, demonstrating robust performance with high precision and accuracy. Identification of related substances was achieved through systematic chromatographic separation, aided by method-specific parameters optimized for specificity and sensitivity. The developed method underwent validation according to ICH guidelines.

Determination of absorption maxima:
The absorbance maxima for lacosamide and any impurities were identified, with shifts in absorbance noted. Following the analysis of the spectra, 210 nm was determined to be the optimal wavelength for spectral analysis and HPLC method development, as it closely aligned with the absorbance maxima for both LA and BA [22].

Selection and optimization of suitable mobile phase for analysis
After performing various trials to scout the mobile phase, Methanol: Water (80:20) was found to provide adequate and acceptable Rt, NTP, and SF for further resolution. Additionally, this mobile phase demonstrated effective peak separation and adequate retention time for the impurity BA, making it suitable for our analysis [23].

Table 1. Optimization of Mobile Phase Ratios

Sr No.	Mobile Phase	Rt (min)	NTP	SF
1	Methanol	4.36	9781	1.080
2	Methanol: Water (90:10)	2.78	4869	1.618
3	Methanol: Water (80:20)	3.31	7093	1.130
4	Methanol: Water (70:30)	4.21	8097	1.010
5	Methanol: Water (60:40)	2.77	4869	1.618
6	Methanol: Water (50:50)	2.78	3706	1.418
7	Methanol: Water (40:60)	2.77	4869	1.618
8	Methanol: Water (30:70)	4.21	8097	1.010
9	Methanol: Water (20:80)	2.78	4869	1.618
10	Methanol: Water (10:90)	2.75	3869	1.518

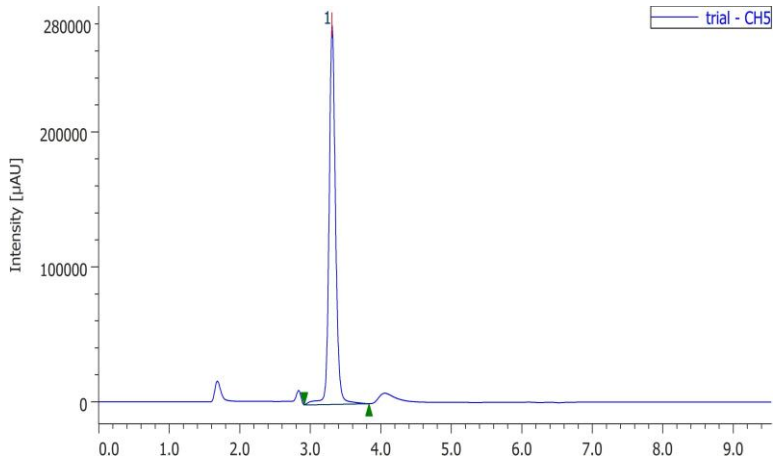


Figure 1. Chromatogram of Lacosamide in Methanol: Water (80: 20 v/v)

System suitability

To assess the suitability of the chromatographic system for accurate quantification of lacosamide and benzyl acetate, several key parameters were evaluated. Retention time indicates the time elapsed from injection to detection of the peak for each compound. The number of theoretical plates (NTP) reflects the efficiency of the column in separating the compounds, with higher values indicating better resolution. Symmetry factor (SF) assesses the peak shape, with values close to 1 indicating symmetrical peaks, which are desirable for accurate quantification. In this study, lacosamide exhibited a retention time of 3.263 minutes, 3609 theoretical plates, and a symmetry factor of 1.644. For benzyl acetate, the retention time was 4.050 minutes, with 2103 theoretical plates and a symmetry factor of 1.01. These results indicate that the chromatographic system provides efficient separation and symmetrical peak shapes, meeting the criteria for reliable quantification of both lacosamide and benzyl acetate [24].

Table 2. Result of System suitability for LA+BA

Parameter	Lacosamide	Benzyl Acetate
Retention Time (min.)	3.263	4.050
Number of Theoretical Plates (NTP)	3609	2103
Symmetry Factor (SF)	1.644	1.01

These results confirm the chromatographic system's suitability for precise and reliable quantification of lacosamide and benzyl acetate, ensuring accurate analytical performance in this study.

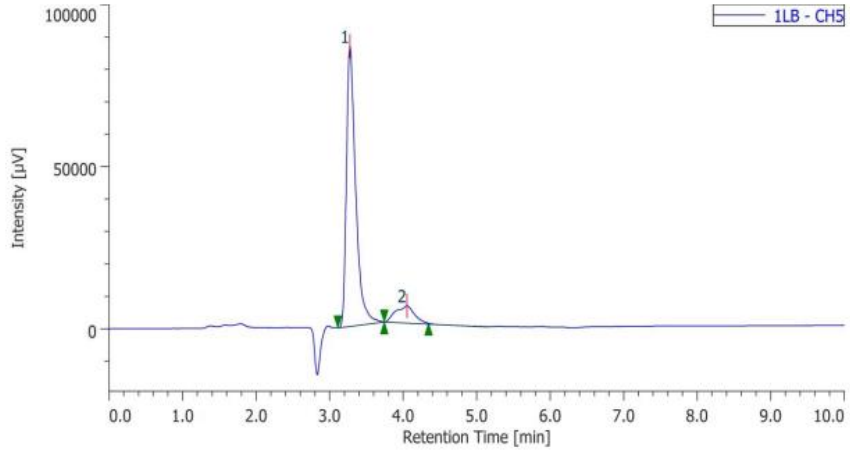


Figure 2. Chromatogram of System suitability for LA+BA

Linearity and range:

In this study, the linearity of the analytical method was evaluated, which assesses its ability to generate test results that correlate proportionally with the concentration of the analyte in the sample. Various concentrations of lacosamide ranging from 2 to 10 µg/ml and benzyl acetate from 0.002 to 0.01 µg/ml were prepared to estimate the method's linearity. This approach ensures the method's capability to accurately quantify different concentrations of lacosamide and benzyl acetate across a specified range, essential for reliable analytical performance in subsequent analyses and applications [25].

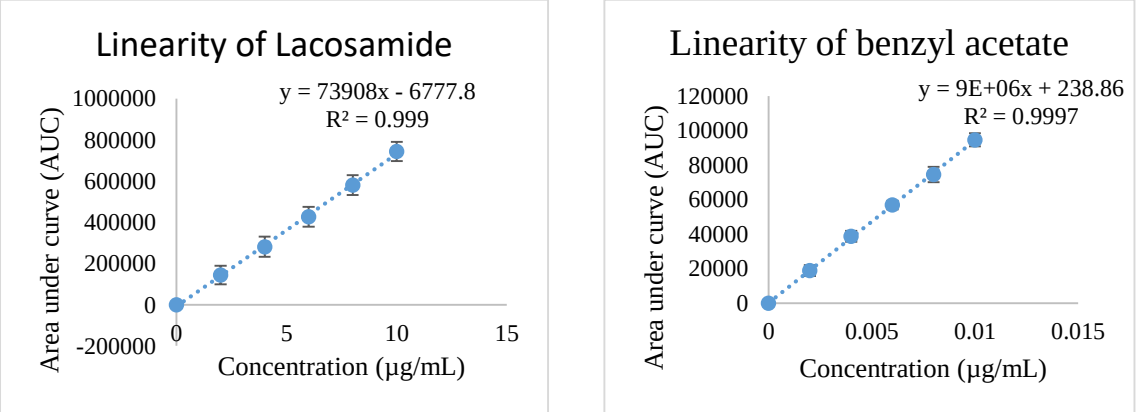


Figure 3. Linearity of (A) Lacosamide (B) Benzyl Acetate

Accuracy:

To assess the accuracy of the analytical method for quantifying lacosamide and benzyl acetate, recovery studies were conducted at various levels of drug concentration. For lacosamide, the accuracy was evaluated at 80%, 100%, and 120% of the target concentration, with corresponding drug recovery calculated as the mean percentage recovery along with the relative standard deviation (RSD). Similarly, for benzyl acetate, accuracy was assessed at 60%, 100%, and 140% levels. The results are presented in the tables below. These data validate the method's accuracy, demonstrating consistent recovery rates across different concentration levels [26].

Table 3. Accuracy of lacosamide

Level of recovery	Conc of Drug (µg/ml)	Conc of pure drug added (µg/ml)	Area under curve (µV˙sec)	Conc of Drug recovered (µg/ml)	% drug recovery	% drug recovery mean ±% RSD
80%	2.5	2	289013	3.96	88.00	87.48±0.462592
			286835	3.93	87.33	
			286135	3.92	87.11	
100%	2.5	2.5	365033	5	100.00	100.133 ± 0.231
			364995	5	100.00	
			366845	5.02	100.4	
120%	2.5	3	435657	5.96	108.36	107.818±1.44
			426688	5.84	106.18	
			437731	5.99	108.90	

Table 4. Accuracy of benzyl acetate

Level of recovery	Conc of Drug (µg/ml)	Conc of pure drug added (µg/ml)	Area under curve (µV˙sec)	Conc of Drug recovered (µg/ml)	% drug recovery	% drug recovery mean ± % RSD
60%	0.0025	0.0015	36567	0.004	100	99.17 ± 1.443
			36064	0.004	100	
			35760	0.0039	97.5	
100%	0.0025	0.0025	45376	0.005	100	100 ± 2
			44885	0.0049	98	
			45948	0.0051	102	
140%	0.0025	0.0035	54598	0.0061	101	99.20 ± 1.559
			53868	0.0059	98.3	
			53855	0.0059	98.3	

Precision

Repeatability (Intra-day precision)

To ensure the precision and consistency of the analytical method, repeatability studies were conducted for both lacosamide and benzyl acetate at different times of the day: morning, afternoon, and evening. These studies involved analyzing multiple concentrations of each analyte and measuring the area under the curve (AUC) to determine the actual concentration and percentage concentration of the analyte. The repeatability is expressed as the mean percentage concentration along with the relative standard deviation (RSD), which indicates the variability in the measurements. The data from these studies provide evidence of the method's robustness and its ability to produce reliable results across different times of the day [27].

Table 5. Repeatability study of Lacosamide

Sr. No.		Conc of Analyte (µg/ml)	Area under curve (µV·sec)	Actual conc of analyte (µg/ml)	% conc of analyte	% conc mean ± % RSD
MORNING	1	6	441631	6.05	100.8	101.64 ± 1.58
	2	8	590610	8.09	101.12	
	3	10	751870	10.3	103	
AFTER-NOON	1	6	446631	6.12	102	100.74 ± 1.47
	2	8	590610	8.09	101.12	
	3	10	723870	9.91	99.10	
EVENING	1	6	446631	6.12	102	102.04 ± 0.90
	2	8	590610	8.09	101.12	
	3	10	751870	10.3	103	

Table 6. Repeatability study of Benzyl Acetate

Sr. No.		Conc of Analyte (µg/ml)	Area under curve (µV·sec)	Actual conc of analyte (µg/ml)	% conc of analyte	% conc mean ± % RSD
MORNING	1	0.006	56852	0.0063	105	103.41 ± 1.87
	2	0.008	73236	0.0081	101.25	
	3	0.010	94211	0.0104	104	
AFTER-NOON	1	0.006	54912	0.0061	101	102.08 ± 1.63
	2	0.008	72936	0.0081	101.25	
	3	0.010	94211	0.0104	104	
EVENING	1	0.006	56852	0.0063	105	103.00 ± 1.76
	2	0.008	72596	0.0080	100	
	3	0.010	95021	0.0105	105	

Intermediate precision

The reproducibility of the analytical method was assessed through intermediate precision studies conducted on two separate days for both lacosamide and benzyl acetate.

Intermediate precision evaluates the method's stability when analyses are conducted under varying conditions, such as different days, analysts, or equipment and provides valuable insights into the method's reliability and consistency over time, verifying its effectiveness for routine analytical use [28].

Table 7. Intermediate precision of Lacosamide

Sr. No.		Conc of Analyte (µg/ml)	Area under curve (µV·sec)	Actual conc of analyte (µg/ml)	% conc of analyte	% conc mean ± % RSD
DAY 1	1	6	441631	6.05	100.8	101.64 ± 1.55
	2	8	590610	8.09	101.125	
	3	10	751870	10.3	103	
DAY 2	1	6	446631	6.12	102	102.04 ± 0.90
	2	8	590610	8.09	101.125	
	3	10	751870	10.3	103	

Table 8. Intermediate precision of Benzyl Acetate

Sr. No.		Conc of Analyte (µg/ml)	Area under curve (µV·sec)	Actual conc of analyte (µg/ml)	% conc of analyte	% conc mean ± % RSD
DAY 1	1	0.006	56852	0.0063	105	103 ± 1.76
	2	0.008	72596	0.0080	100	
	3	0.010	95021	0.0105	105	
DAY 2	1	0.006	54912	0.0061	101	102.08 ± 1.63
	2	0.008	72936	0.0081	101.25	
	3	0.010	94211	0.0104	104	

LOD and LOQ determination

The sensitivity of the analytical method was evaluated by determining the LOD and LOQ for LA and BA. These parameters indicate the lowest concentrations at which the analyte can be reliably detected and quantified, respectively. The results for LOD and LOQ for both lacosamide and benzyl acetate are summarized in the table below, demonstrating the method's high sensitivity and suitability for detecting and quantifying these compounds at low concentrations [29].

Table 9. Results of LOD and LOQ of lacosamide and benzyl acetate

Parameters	Lacosamide	Benzyl acetate
LOD	0.4191	0.00083157
LOQ	1.2700	0.00027442

Robustness

The robustness of the analytical method was evaluated by examining its performance under slight variations in critical parameters. Robustness testing assesses the method's reliability and consistency when subjected to small, deliberate changes in flow rate, mobile phase composition, and detection wavelength. The findings, detailed in the table below, demonstrate the method's robustness and its capacity to maintain accuracy and precision despite minor changes in experimental conditions [13].

Table 10. Results of robustness study of lacosamide

Parmeters		Conc of Analyte (µg/ml)	Area under curve (µV·sec)	Actual conc of analyte (µg/ml)	% conc of analyte	% conc mean ± % RSD
Change in flow rate (ml/min)	0.9	6	436737	5.98	99.66	99.58 ± 0.113
	1.1	6	435934	5.97	99.50	
Change in mobile phase composition	79:21	6	436433	5.98	99.66	99.58 ± 0.113
	81:19	6	435657	5.97	99.50	
Change in detection wavelength (nm)	209	6	436688	5.98	99.66	99.745 ±0.120
	211	6	437731	5.99	99.83	

Specificity

The specificity of the method was assessed by confirming that the lacosamide peak from the marketed formulation was distinct and free from interference with benzyl acetate or other formulation components, validating the method's ability to accurately identify lacosamide in complex matrices [14].

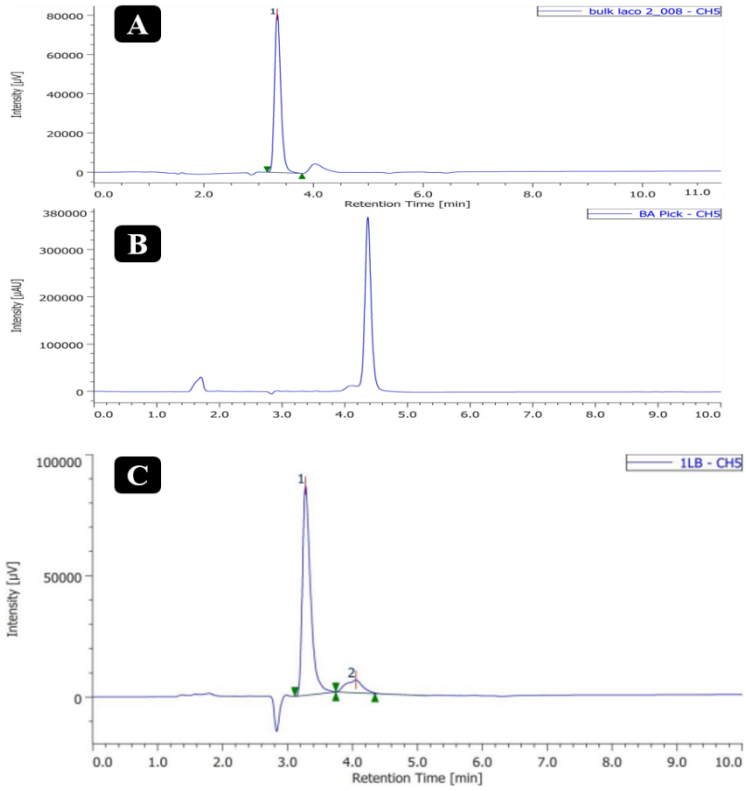


Figure 4. Overlain spectra of (A) Plain lacosamide (B) Plain Benzyl acetate (C) Marketed formulation

Short-term stability study

This study is crucial as it assesses the robustness of the analytical method under ambient conditions, reflecting real-world scenarios where sample solutions may need to remain stable for extended periods before analysis. The results give in table below highlight the method's reliability in maintaining consistent analytical characteristics for both lacosamide and benzyl acetate over the specified time frame [15].

Table 11. Short term stability study

Parameter	Time (t=0)		Time (t=24 hrs)	
	Lacosamide	**Benzyl Acetate**	**Lacosamide**	**Benzyl Acetate**
Retention Time (min.)	3.263	4.050	3.259	4.078
Number of Theoretical Plates (NTP)	3609	2103	3613	2101
Symmetry Factor (SF)	1.644	1.010	1.689	1.006

4. Conclusion

This study successfully developed and validated a novel HPLC method for the quantification of Lacosamide (LA) and its primary impurity, benzyl acetate (BA). The method demonstrated high precision, accuracy, and robustness, making it a reliable tool for therapeutic drug monitoring and quality control in pharmaceutical formulations. Key advantages of the method include reduced sample preparation time, enhanced sensitivity, and comprehensive impurity profiling, which are critical for ensuring drug safety and efficacy.

The method's performance metrics, such as high linearity ($R^2 = 0.9998$ for LA and $R^2 = 0.9995$ for BA), excellent recovery rates (98.5% to 101.2%), and low detection limits (0.03 µg/mL for BA), highlight its analytical strength. Compared to existing methodologies, this novel method offers improved sensitivity, efficiency, and robustness, aligning with regulatory requirements and industry standards.

The study underscores the importance of advanced analytical methods in pharmaceutical analysis, particularly in the context of LA, a crucial antiepileptic drug. Reliable quantification and impurity profiling are essential for optimizing therapeutic efficacy and ensuring patient safety. The novel HPLC method developed in this study addresses these needs, providing a valuable tool for both clinical and pharmaceutical applications.

Future work will focus on further optimizing the method for high-throughput applications and exploring its potential for analyzing other antiepileptic drugs. Additionally, integrating automated sample preparation techniques could enhance the method's efficiency and applicability in routine clinical and pharmaceutical settings. Overall, this validated HPLC method represents a significant advancement in the analytical quantification of Lacosamide and its impurities, contributing to improved clinical outcomes and patient safety.

5. References

1. *Contin M, Albani F, Riva R, Candela C, Mohamed S, Baruzzi A. Lacosamide therapeutic monitoring in patients with epilepsy: effect of concomitant antiepileptic drugs. Therapeutic drug monitoring. (**2013**) Dec 1;35(6):849-52. https://doi.org/10.3390/analytica2040016*

2. *Curia G, Biagini G, Perucca E, Avoli M. Lacosamide: a new approach to target voltage-gated sodium currents in epileptic disorders. CNS drugs. **(2009)** Jul;23:555-68.*

3. *Johannessen SI, Battino D, Berry DJ, Bialer M, Krämer G, Tomson T, Patsalos PN. Therapeutic drug monitoring of the newer antiepileptic drugs. Therapeutic drug monitoring. **(2003)** Jun 1;25(3):347-63. DOI: 10.1097/00007691-200306000-00016*

4. *Lambert MJ, Hansen NB, Finch AE. Patient-focused research: using patient outcome data to enhance treatment effects. Journal of consulting and clinical psychology. **(2001)** Apr;69(2):159. https://doi.org/10.1037/0022-006X.69.2.159*

5. *Dhangar KR, Jagtap RB, Surana SJ, Shirkhedkar AA. Impurity profiling of drugs towards safety and efficacy: theory and practice. Journal of the Chilean Chemical Society. **(2017)** Jun;62(2):3543-57. http://dx.doi.org/10.4067/S0717-97072017000200024*

6. *Wu Y, Levons J, Narang AS, Raghavan K, Rao VM. Reactive impurities in excipients: profiling, identification and mitigation of drug–excipient incompatibility. Aaps Pharmscitech. **(2011**) Dec;12:1248-63. https://doi.org/10.1208/s12249-011-9677-z*

7. *Mandrioli R, Protti M, Marincich L, Mercolini L. Ion-Channel Antiepileptic Drugs: An Analytical Perspective on the Therapeutic Drug Monitoring (TDM) of Ezogabine, Lacosamide, and Zonisamide. Analytica. **(2021)** Nov 30;2(4):171-94. https://doi.org/10.3390/analytica2040016*

8. *Jalakam SP, Tambe VS, Deodhar MN, Prakya V, Waghmode J, Pawar P. Direct Chiral HPLC-MS/MS Method for Determination of R-Lacosamide in Human Plasma. Pharmaceutical Chemistry Journal. **(2020)** Apr;54(1):90-7. https://doi.org/10.1007/s11094-020-02162-6*

9. *Vadagam N, Haridasyam SB, Venkatanarayana M. Stability-indicating normal-phase HPLC method development for separation and quantitative estimation of S-enantiomer of lacosamide in pharmaceutical drug substance and tablet dosage form. Biomedical Chromatography. **(2023)** Sep;37(9):e5688. https://doi.org/10.1002/bmc.5688*

10. *Greenaway C, Ratnaraj N, Sander JW, Patsalos PN. A high-performance liquid chromatography assay to monitor the new antiepileptic drug lacosamide in patients with epilepsy. Therapeutic drug monitoring. **(2010)** Aug 1;32(4):448-52. DOI: 10.1097/FTD.0b013e3181dcc5fb*

11. *Chakravarthy VK, Sankar DG. Stability indicating Hplc method for determination of Lacosamide and its degradants/impurities in bulk and pharmaceutical formulation. Rasayan Journal of Chemistry. **(2012)**;5:293-310.*

12. Jane J, Ladani D. Development and Validation of Analytical Methods for Lacosamide. Research Journal of Pharmacy and Technology. ***(2013)****;6(5):553-5.*

13. Chhalotiya UK, Bhatt KK, Shah DA, Baldania SL, Patel JR. Stability-indicating liquid chromatographic method for quantification of new anti-epileptic drug lacosamide in bulk and pharmaceutical formulation. Chemical Industry and Chemical Engineering Quarterly. ***(2012)****;18(1):35-42. https://doi.org/10.2298/CICEQ110821044C*

14. Sonawane J, Rathod S, Suryawanshi S, Kale D, Chaudhari A. Development and validation of RP-HPLC method for estimation of lacosamide in bulk and its pharmaceutical formulation. Asian Journal of Pharmaceutical Analysis. ***(2022)****;12(1):23-8. DOI : 10.52711/2231-5675.2022.00005*

15. Gonçalves J, Alves G, Bicker J, Falcão A, Fortuna A. Development and full validation of an innovative HPLC-diode array detection technique to simultaneously quantify lacosamide, levetiracetam and zonisamide in human plasma. Bioanalysis. ***(2018)*** *Apr;10(8):541-57. https://doi.org/10.4155/bio-2017-0199*

16. Kestelyn C, Lastelle M, Higuet N, Dell'Aiera S, Staelens L, Boulanger P, Boekens H, Smith S. A simple HPLC–UV method for the determination of lacosamide in human plasma. Bioanalysis. ***(2011)*** *Nov 1;3(22):2515-22. https://doi.org/10.4155/bio.11.261*

17. 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.*

18. Chakravarthy VK, Shankar DG. HPLC method for determination of lacosamide S (−) enantiomer in bulk and pharmaceutical formulation. Rasayan Journal of Chemistry. ***(2011)****;4(4):744-52.*

19. Löscher W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure. ***(2011)*** *Jun 1;20(5):359-68.*

20. Rana R, Sharma R, Kumar A, Kumar S. Advances in Ultra HPLC-MS: Unraveling the Bioanalytical Landscape of Drug Detection and Metabolite Profiling.

21. D'Atri V, Fekete S, Clarke A, Veuthey JL, Guillarme D. Recent advances in chromatography for pharmaceutical analysis. Analytical chemistry. ***(2018)*** *Nov 8;91(1):210-39.*

22. Iftequar S, Swaroop L, Zaheer Z, Shahid M, Imran S, Dehghan MH. UV Spectrophotometric methods for estimation of Ramipril in Pharmaceutical dosage form by absorption maxima method and area under curve. Int. J. Drug Dev. & Res. ***(2012)*** *Jan;4(1):286-90.*

23. Yadlapalli SS, Katari NK, Manabolu Surya SB. Determination of lacosamide in human plasma by LC–MS/MS and its application to a human pharmacokinetic study. Separation Science Plus. ***(2020)*** *Jun;3(6):216-24.*

24. Bharwad KD, Shah PA, Shrivastav PS, Sharma VS. Selective quantification of lacosamide in human plasma using UPLC–MS/MS: Application to pharmacokinetic study in healthy subjects with different doses. Biomedical Chromatography. ***(2020)*** *Nov;34(11):e4928.*

25. Peng Y, Luo J, Lu Q, Chen X, Xie Y, Chen L, Yang W, Du S. HPLC analysis, semi-preparative HPLC preparation and identification of three impurities in salidroside bulk drug. Journal of pharmaceutical and biomedical analysis. ***(2009)*** *Apr 5;49(3):828-32. https://doi.org/10.1016/j.jpba.2008.12.025*

26. Kowalska M, Woźniak M, Kijek M, Mitrosz P, Szakiel J, Turek P. Management of validation of HPLC method for determination of acetylsalicylic acid impurities in a new pharmaceutical product. Scientific Reports. ***(2022)*** *Jan 6;12(1):1. https://doi.org/10.1038/s41598-021-99269-x*

27. Marson BM, Concentino V, Junkert AM, Fachi MM, Vilhena RO, Pontarolo R. Validation of analytical methods in a pharmaceutical quality system: An overview focused on HPLC methods. Química Nova. ***(2020)*** *Oct 16;43:1190-203. https://doi.org/10.21577/0100-4042.20170589*

*28. Bulduk İ. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta Chromatographica***.** ***(2021)*** *Apr 28;33(3):209-15. https://doi.org/10.1556/1326.2020.00828*

29. Al-Hakkani MF, Gouda GA, Hassan SH, Farghaly OA, Mohamed MM. Fully investigation of RP-HPLC analytical method validation parameters for determination of Cefixime traces in the different pharmaceutical dosage forms and urine analysis. Acta Pharmaceutica Sciencia. ***(2021)****;59(1).*

6. Acknowledgement:

The authors would like to express their sincere gratitude to Bharati Vidyapeeth College of Pharmacy for providing the necessary facilities and resources to conduct this research. We also thank Clearsynth Pvt. Ltd. for supplying the reference standard of Lacosamide. We extend our appreciation to our colleagues in the Department of Pharmaceutical Quality Assurance for their invaluable support and technical assistance throughout the study.