Journal of Molecular Science

Journal of Molecular Science
Volume 36 Issue 1, Year of Publication 2026, Page 210-217
DoI-10.004687/1000-9035.2026.026

Journal of Molecular Science

www.jmolecularsci.com ISSN:1000-9035

Analytical Method Development and Validation for Simultaneous Estimation of Lacosamide Oxalate and Ibuprofen Sodium in Synthetic Mixture

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Article Information
Received: 16-12-2025
Revised: 21-01-2026
Accepted: 18-02-2026
Published: 26-03-2026

Keywords
Lacosamide Oxalate (LACO); Ibuprofen Sodium (IBU); High-performance liquid chromatography (HPLC) and UV spectrophotometric methods (UV).

ABSTRACT
Lacosamide oxalate, an antiepileptic drug, and Ibuprofen sodium, a nonsteroidal anti-inflammatory drug (NSAID), exhibit distinct pharmacological mechanisms and may provide potential therapeutic benefits when used in combination, particularly for neurological conditions associated with pain such as Episodic migraine. The present study aimed to develop and validate simple, rapid, accurate, and precise analytical methods for the simultaneous estimation of Lacosamide oxalate and Ibuprofen sodium in a synthetic mixture. Two analytical techniques were employed as UV-spectrophotometric method using first-order derivative spectroscopy and a reverse-phase high-performance liquid chromatography (RP-HPLC) method. In the UV method, the zero-crossing point (ZCP) of Lacosamide oxalate and Ibuprofen sodium were found to be 274 nm and 220 nm, respectively. Linearity was observed in the range of 1-5 µg/ml at 220 nm for Lacosamide oxalate and 8-40 µg/ml at 274 nm for Ibuprofen sodium. The RP-HPLC method was developed using a mobile phase of acetonitrile and phosphate buffer (pH 3 adjusted with 10% ortho phosphoric acid) in a ratio of 55:45 % v/v, with detection at 212 nm. The retention time were 3.0 min for Lacosamide oxalate and 5.2 min for Ibuprofen sodium. Both methods were validated according to ICH Q2 (R2) guideline. For both method Linearity was observed in the range of 1-5 µg/mL for Lacosamide oxalate and 8-40 µg/mL for Ibuprofen sodium, with correlation coefficients greater than 0.998. Precision studies showed % RSD values below 2%. Accuracy, determined by recovery studies, ranged between 98% to 101% for both drugs. The methods also demonstrated low limits of detection sand quantification, indicating good sensitivity. In conclusion, both developed and validated methods found to be accurate, economical and Reproducible. There was no interference of any Excipients in the determination of Drugs from synthetic mixture.

1. INTRODUCTION:

The combination of Lacosamide oxalate and Ibuprofen sodium represents a rationale therapeutic approach based on complementary mechanisms of action. Lacosamide oxalate is an antiepileptic drug (AED) used to treat partial-onset seizures in adults and children[1]. Epilepsy is a group of non-communicable neurological disorders characterized by recurrent epileptic seizures. An epileptic seizure is the clinical manifestation of an abnormal, excessive, and synchronized electrical discharge in the neurons. It works by stabilizing hyperexcitable neuronal membranes and enhancing slow

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inactivation of voltage-gated sodium channels[2]. This helps reduce excessive electrical activity in the brain that can lead to seizures[3-4]. Ibuprofen sodium is a nonsteroidal anti-inflammatory drug (NSAID) used to relieve pain, fever, and inflammation. It works by inhibiting cyclooxygenase (COX-1 and COX-2) enzymes, which reduces the production of prostaglandins, chemicals involved in pain and inflammation[5]. Mixture of Lacosamide oxalate and Ibuprofen sodium is used to treat the episodic Migraine. Episodic Migraine is a type of migraine characterized by headache attacks that occur on fewer than 15 days per month. These attacks are typically moderate to severe in intensity, often accompanied by symptoms such as nausea, vomiting, and sensitivity to light and sound. Unlike chronic migraine, which occurs on 15 or more days per month, episodic migraine is less frequent but can still significantly impact daily life[6-7]. Combination of Lacosamide oxalate and Ibuprofen sodium was studied under clinical trial phase 3[6]. It was proved that therapy improved to prevention of episodic migraine in patient. The combination together can be used for the treatment aged 10-55-year suffering from headache disorder like migraine[7]. Literature survey reveals UV spectrophotometric [8-9], RP-HPLC [10-14], thin layer chromatography-densitometry[15], stability-indicating HPLC[16, 17], GC-MS[18], UPLC-MS/MS[19, 20], HPLC-UV[21-23] method for the estimation of both drugs alone and in combinations. From extensive literature survey, there is no any analytical method available in this combination. Given the widespread use of Lacosamide oxalate and Ibuprofen sodium in the management of Episodic Migraine, the development of validated analytical methods for their simultaneous estimation is essential to ensure quality, safety, and efficacy of this combination intended for large patient populations. The objectives of the present work were to develop and validate a linear, accurate, precise, and sensitive first-order derivative UV method and RP-HPLC method for the simultaneous estimation of Lacosamide oxalate and Ibuprofen sodium in synthetic mixture, providing a practical and robust solution for routine laboratory applications. These methods were validated according to ICH Q2 (R2) guideline[24] within all Validation Parameters.

2. MATERIALS AND ANALYTICAL CONDITIONS:

2.1 Reagents and chemicals:

Lacosamide oxalate (Mekanar Pharma, Hyderabad, India), Ibuprofen sodium (Intas Pharmaceutical, Ahmedabad, India) was received as gift sample. Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade) (Finar Chemicals Pvt. Ltd.-Ahmedabad), Ortho Phosphoric Acid 75 % (AR Grade) (Astron Chemical India). All other reagents employed were of high purity analytical grade. All weighing was done on a calibrated analytical balance. Calibrated glassware's were used throughout the work.

2.2 Instruments & Software:

The spectrophotometric measurements were performed using a UV-Visible spectrophotometer (Shimadzu-1900, UV Probe 2.7 version software) with a spectral bandwidth of 1 nm was employed for all spectroscopic measurements, using a pair of 1.0 cm matched quartz cells over the range of 200-400 nm. For chromatographic information acquisition and analysis, High-Performance Liquid Chromatography system Systronic RP-HPLC (LC-20-AD) (SPD-20 A) with UV Detector was utilized together. The pH of the buffer solution was observed utilizing the Chemi Line pH meter. The Scale-Tec analytical balance was utilized to weigh the samples. The HPLC mobile phase was subjected to sonication using an Sonicator- Digital Pro$^+$ (PS 10A).

2.3 Analytical conditions:

In accordance with ICH $Q_2(R_2)$ guideline[24] requirements, the analytical conditions for a First order derivative technique for the measurement of Lacosamide oxalate and Ibuprofen sodium in UV and RP-HPLC were optimized and validated. For UV Spectroscopy Methanol was used as a Solvent. Detection wavelength (λmax) of LACO and IBU 215 nm and 221 respectively. The first-order derivative UV spectra were derived from the zero-order spectra using methanol as the solvent. Quantitative analysis was performed at the zero-crossing point (ZCP) of Lacosamide oxalate at 274 nm for the estimation of Ibuprofen sodium, and at the ZCP of Ibuprofen sodium at 220 nm for the estimation of Lacosamide oxalate. For RP-HPLC, Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) Column, ACN: Phosphate Buffer (pH 3): Methanol (55:45 % v/v) used as mobile phase at 1 ml/min flow rate. 212 nm was selected for HPLC method using U.V Detector. 20 µL injection volume was injected by using Hamilton syringe.

2.4 *Preparation of Solutions:*

2.4.1 Preparation of Stock Solution:

Accurately weighed 10 mg of Lacosamide oxalate and 10 mg of Ibuprofen sodium were individually transferred into separate 100 mL volumetric flasks and dissolved in methanol. The solutions were sonicated to ensure complete dissolution, and the volume was made up to the mark with methanol to obtain standard stock solutions having a concentration of Lacosamide oxalate 100 µg/mL of and 100 µg/mL of Ibuprofen sodium, respectively.

2.4.2 *Preparation standard solution:*

Accurately 0.04 ml of the [mixture solution of

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Lacosamide oxalate (500 µg/ml) and Ibuprofen sodium (4000 µg/ml)] was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Lacosamide oxalate was 2 µg/ml and Ibuprofen sodium 16 µg/ml.

2.4.3 Preparation of standard working solution:
The concentration ranges of Lacosamide oxalate (100 µg/ml) (0.1, 0.2, 0.3, 0.4 and 0.5 ml) and Ibuprofen sodium (100 µg/ml) (0.8, 1.6, 2.4, 3.2 and 4.0 ml) were pipetted out in five different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of about 1, 2, 3, 4 and 5 µg/ml for Lacosamide oxalate and 8, 16, 24, 32 and 40 µg/ml for Ibuprofen sodium. Under the optimized spectrophotometric conditions, the samples were analysed using a 1 cm quartz cuvette in the UV spectrophotometer. Similarly, the optimized chromatographic conditions, 20 µL of each standard working solution were injected into RP-HPLC system by Hamilton syringe and analysed.

3. METHODOLOGY:

3.1 UV-spectrophotometric method:
A first-order derivative spectrophotometric technique was employed for the simultaneous quantification of Lacosamide oxalate and Ibuprofen sodium in a synthetic mixture. Separate working standard solutions of each drug were scanned within the 200-400 nm wavelength range to generate their derivative spectra, enabling the determination of appropriate zero-crossing wavelengths for accurate quantitative analysis. Lacosamide oxalate and Ibuprofen sodium standard stock solutions were prepared in Methanol at concentrations of 100 µg/mL and 100 µg/mL, respectively. Pipetted out 0.1 ml solution from stock solution of Lacosamide oxalate (100 µg/ml) and 0.8 ml Ibuprofen sodium (100 µg/ml) into different 10 ml volumetric flask and diluted up to mark with Methanol to get the 1 µg/ml of Lacosamide oxalate and 8 µg/ml Ibuprofen sodium. Each solution was scanned in the range of 200-400 nm. All zero-order spectrum (D^0) were converted to first derivative spectrum (D^1) using delta lambda 2.0 and scaling factor 4. The overlain first derivative spectrums of Lacosamide oxalate and Ibuprofen sodium at different concentration were recorded. The zero-crossing point (ZCP) of Lacosamide oxalate and Ibuprofen sodium were found to be 274 nm and 220 nm, respectively. The zero order and first order overlay UV spectra of Lacosamide oxalate and Ibuprofen sodium showed in Figure 1 and 2, respectively.

3.2. Reverse Phase High Performance Liquid Chromatography Method:
For RP-HPLC, the analysis was carried out using an isocratic elution technique using a mobile phase comprised of different mobile phases such as ACN: Phosphate Buffer (pH 3 adjusted with 10% ortho phosphoric acid) (55:45 % v/v) at a flow rate of 1 mL/min found better separation of both the drug peaks. Prior to usage, the solvents were filtered through a 0.45 µm filter and sonicated for 30 min. The stationary phase was a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm), and the eluent was observed by a U.V Detector from 200 to 400 nm, alongside chromatograms extracted at 212 nm. The calibration curves were prepared by measuring the peak areas of LACO and IBU and plotted their values against the pertinent concentrations. In accordance, the equations for linear regression were calculated.

3.3 Method Validation:
The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) $Q_2(R_2)$[22]: Validation of Analytical Procedures established standards for the validation of the analytical procedures utilized in this investigation.

3.3.1 Specificity:
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

3.3.2. Linearity and Range (n=6):
The linearity of Lacosamide oxalate and Ibuprofen sodium were found to be in the range of 1-5 µg/ml and 8-40 µg/ml respectively. Plot the calibration curve of Peak area Vs Concentration (µg/ml). Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

3.3.3. Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, Reproducibility (Interday precision), Repeatability.

i. **Intraday Precision (n=3):** Solutions containing 1, 2, 3 µg/ml of Lacosamide oxalate and 8, 16, 24 µg/ml of Ibuprofen sodium were analyzed three times on the same day and % R.S.D was calculated.
ii. **Interday Precision (n=3):** Solutions containing 1, 2, 3 µg/ml of Lacosamide oxalate and 8, 16, 24 µg/ml of Ibuprofen sodium were analyzed on three different successive days and % R.S.D was calculated.
iii. **Repeatability (n=6):** Solutions containing 2

µg/ml of Lacosamide oxalate and 16 µg/ml of Ibuprofen sodium were analyzed for six times and % R.S.D. was calculated.

3.3.4 Limit of Detection (LOD):

Limit of detection can be calculated using following equation as per ICH $Q_2(R_2)$ guideline[22].

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve

S = slope of the calibration curve

3.3.5 Limit of Quantification (LOQ):

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH $Q_2(R_2)$ guideline[22].

$$\mathrm{LOQ} = 10 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve

S = slope of the calibration curve

3.3.6. Accuracy (Recovery study) (n=3)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH $Q_2(R_2)$ guideline[22] at three different concentration levels 50 %, 100 %, 150 % and the values were measured for Lacosamide oxalate (2 µg/ml) and Ibuprofen sodium (16 µg/ml). This performance was done in triplicate. The accuracy of the method was determined by calculating recovery of Lacosamide oxalate and Ibuprofen sodium by the standard addition method.

3.3.7. Assay as analysis of Synthetic Mixture:

The synthetic mixture of Lacosamide oxalate and Ibuprofen sodium was prepared in the ratio of 50:400. Accurately weighed equivalently weight of Lacosamide oxalate (50 mg) and Ibuprofen sodium (400 mg) and transferred in 100 ml volumetric flask and allow to sonicate and made up to mark with Methanol. Common excipients such as MCC (Micro Crystalline Cellulose) (15 mg), Starch (10 mg), Magnesium stearate (10 mg), Talc (10 mg) and Croscarmellose Sodium (5 mg) were added in the motor pestle along with the drug Lacosamide oxalate (50 mg) and Ibuprofen sodium (400 mg). This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 500 µg/ml of Lacosamide oxalate and 4000 µg/ml of Ibuprofen sodium.

3.3.7.1 Preparation of sample solution:

Accurately 0.04 ml of the above (mixture solution of Lacosamide oxalate 500 µg/ml and Ibuprofen sodium 4000 µg/ml) was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Lacosamide oxalate was 2 µg/ml and Ibuprofen sodium 16 µg/ml.

3.3.8 Robustness:

The robustness of analytical methods becomes evaluated to decide their ability to face up to minor variations in approach situations. For the HPLC technique, samples have been subjected to evaluation below changed situations, which include adjustments inside the flow rate (± 0.1 mL/min), detection wavelength (± 2 nm), and natural content material (± 2 %) inside the mobile segment. The resulting results on machine suitability parameters have been intently monitored. In the times of Methods I and II, distinct analysts conducted sample analyses to evaluate the robustness of the strategies.

3.3.9 System Suitability Tests:

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates.

4. RESULTS AND DISCUSSION:

4.1 UV SPECTROPHOTOMETRIC METHOD:

The first-order derivative UV spectrophotometric method offers superior selectivity, reduced spectral interference, and improved resolution compared with conventional zero-order UV methods, making it particularly advantageous for First order derivative technique and routine analysis of pharmaceutical formulations.

4.1.1 Selection of wavelength for Lacosamide oxalate Ibuprofen sodium:

Pipetted out 0.1 ml solution from stock solution of Lacosamide oxalate (100 µg/ml) and 0.8 ml Ibuprofen sodium (100 µg/ml) into different 10 ml volumetric flask and diluted up to mark with Methanol to get the 1 µg/ml of Lacosamide oxalate and 8 µg/ml Ibuprofen sodium. Each solution was scanned in the range of 200-400 nm. All zero-order spectrum (D^0) were converted to first derivative spectrum (D^1) using delta lambda 2.0 and scaling factor 4. The overlain first derivative spectrums of Lacosamide oxalate and Ibuprofen sodium at different concentration were recorded. The zero-crossing point (ZCP) of Lacosamide oxalate and Ibuprofen sodium were found to be 274 nm and 220 nm, respectively. Overlain Spectra of Lacosamide oxalate (2 µg/ml) and Ibuprofen sodium (16 µg/ml)

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in Methanol (First order) have been shown in Figure 2.

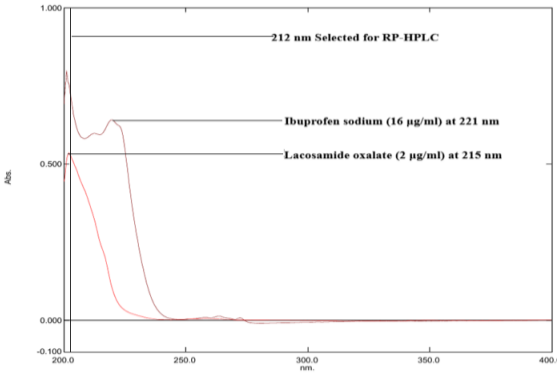

Figure 1: Overlain UV Spectra of Lacosamide oxalate (2 µg/ml) and Ibuprofen sodium (16 µg/ml) in Methanol

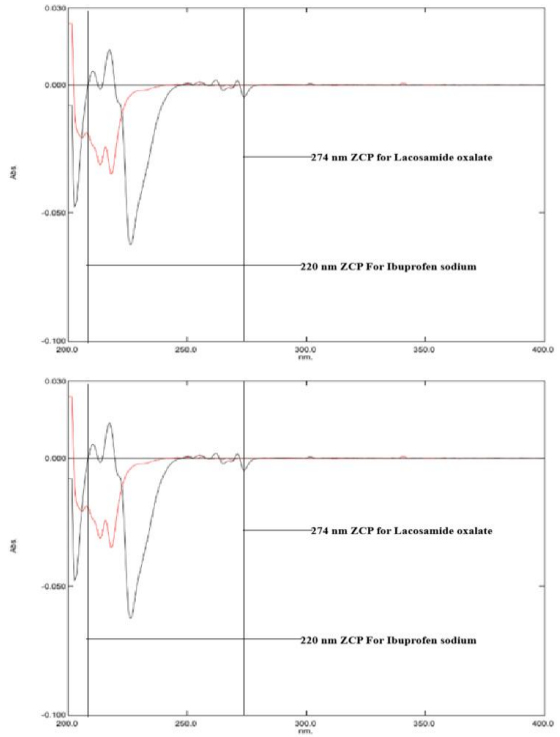

Figure 2: Overlain UV Spectra of Lacosamide oxalate (2 µg/ml) and Ibuprofen sodium (16 µg/ml) in Methanol (First order)

4.2 RP-HPLC METHOD:

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also performs better in terms of efficiency, stability and reproducibility. C_{18} column was selected because it is least polar compare to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds. In addition to this UV detector is used which allows easy detection of the compounds in UV transparent organic solvents. Hence, C_{18} (250×4.6 mm) column of 5 µm particle packing was selected for separation of Lacosamide oxalate and Ibuprofen sodium. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability.

4.2.1 Selection detection wavelength:

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 212 nm both drugs give good peak height and shape. So, 212 nm was selected for simultaneous estimation of Lacosamide oxalate and Ibuprofen sodium in synthetic mixture. Overlain UV spectra of Lacosamide oxalate (2 µg/ml) and Ibuprofen sodium (16 µg/ml) in Methanol has been shown in figure 1. Various mobile phases, such as Methanol: Water, Acetonitrile: Water, Methanol: Buffer in different proportion was tried. The combination of ACN: Phosphate Buffer (pH 3 adjusted with 10% ortho phosphoric acid) (55:45 % v/v) was selected because it was found to ideally resolve the peaks with retention time 3 min and 5.2 min for Lacosamide oxalate and Ibuprofen sodium, respectively. Kromstar C_{18} (250×4.6 mm, 5 µm) column was used for separation of Lacosamide oxalate and Ibuprofen sodium with Flow rate of 1.0 ml/min.

4.3 VALIDATION PARAMETERS OF THE UV METHOD AND RP-HPLC METHOD

4.3.1 Linearity and range

For LACO and IBU, the absorbances ranged from 1-5 µg/ml at 274 nm and 8-40 µg/ml at 220 nm showed in Figure 3 (A) and Figure 3 (B) respectively.

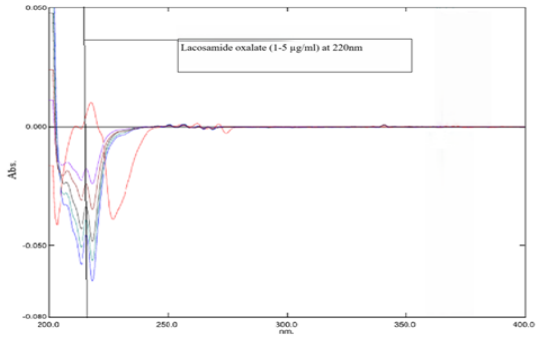

Figure 3 (A): Overlain UV Spectra of Lacosamide oxalate (1-5 µg/ml) at 220 nm

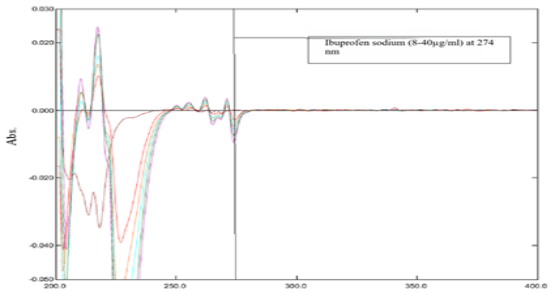

Figure 3 (B): Overlain UV Spectra of Ibuprofen sodium (8-40 µg/ml) at 274 nm

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The UV- Spectroscopy Linearity Range of 1-5 µg/ml for Lacosamide oxalate and 8-40 µg/ml for Ibuprofen sodium. A linear relationship was found and calibration curve was plotted for concentration vs. absorbance. For LACO, the calibration curve equation y =0.0062x + 0.0056, while for IBU, it was y =0.0001x + 0.002. Results showed that the correlation coefficient (R^2) was between 0.999 and 1. The RP-HPLC chromatogram 1-5 µg/ml for Lacosamide oxalate and 8-40 µg/ml for Ibuprofen sodium. A linear relationship was found and calibration curve was plotted for concentration vs. mean peak area. For LACO, the calibration curve equation y = 143.24x - 48.036, while for IBU, it was y = 40.588x - 48.931. Results showed that the correlation coefficient (R^2) was between 0.9994 and 0.998 in RP-HPLC. (Table 1)

Table 1: Linearity data for UV Spectrophotometry & RP-HPLC Method

Parameters	UV Spectrophotometry		RP-HPLC	
	LACO at 220 nm	IBU at 274 nm	LACO at 212 nm	IBU at 212 nm
Linearity Range	1-5 µg/ml	8-40 µg/ml	1-5 µg/ml	8-40 µg/ml
Regression Equation	y =0.0062x + 0.0056	y =0.0001x + 0.002	y = 143.24x - 48.036	y = 40.588x - 48.931
Correlation Coefficient	0.999	1	0.9994	0.998
LOD	0.08	1.55	0.02	0.28
LOQ	0.25	4.65	0.07	0.84

4.3.2 Precision:

In terms of precision, both Inter-day, Intraday and Repeatability measurements were conducted at three distinct concentrations 1, 2, 3 µg/ml of LACO and 8, 16, 24 µg/ml of IBU triplicate over three consecutive days and on the same day. The absorbance of the same solutions was measured. For repeatability, 2µg/ml of LACO and 16µg/ml of IBU were measured. The resulting %RSD values for Intraday, Inter-day precision, and Repeatability were showed in Table 2, respectively.

Table 2: Precision study of LACO & IBU for UV Spectrophotometry & RP-HPLC

Sr. No.	Parameters	UV Spectrophotometry		RP-HPLC	
		LACO at 220 nm	IBU at 274 nm	LACO at 212 nm	IBU at 212 nm
1	Intraday Precision (%RSD, n=3)	1.04-1.34	1.36-1.56	0.87-1.02	0.71 - 1.13
2	Interday Precision (% RSD, n=3)	1.08-1.41	1.32-1.62	0.89-1.06	0.74 - 1.15
3	Repeatability (% RSD, n=6)	1.27	1.40	0.96	0.88

4.3.3 Accuracy

To decide the accuracy of the technique recuperation, change into accomplished by means of standard addition approach. To pre-analysed pattern acknowledged quantity of general LACO and IBU spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of LACO and IBU. Accuracy was carried out by the Recovery Studies (standard addition method). The results were stipulated in triplicate and the accuracy indicated by % recovery. In UV method, The % Recovery was obtained in range of 99.33%-99.80% for LACO and 99.84 % - 99.89 %for IBU. In RP-HPLC method, 99.33%-99.80% for LACO and 99.87%-99.97% for IBU were showed in Table 3.

Table 3: Recovery study for UV Spectrophotometry & HPLC Method

Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Std Amt (µg/ml)	Total amount Recovered (µg/ml)	% Mean Recovery ± SD(n=3)
UV method						
Lacosamide oxalate	50	2	1	3	2.98	99.44± 0.157
	100	2	2	4	3.97	99.25± 0.204
	150	2	3	5	4.96	99.36± 0.259
Ibuprofen sodium	50	16	8	24	23.96	99.84± 0.082
	100	16	16	32	31.96	99.89± 0.034
	150	16	24	40	39.96	99.89± 0.112
RP-HPLC Method						
Lacosamide oxalate	50	2	1	3	2.98	99.33± 1.1014
	100	2	2	4	3.98	99.50± 0.1193
	150	2	3	5	4.99	99.80± 1.1571
Ibuprofen sodium	50	16	8	24	23.97	99.87± 1.1322
	100	16	16	32	31.98	99.93± 1.2328
	150	16	24	40	39.99	99.97± 1.2421

4.3.4 LOD and LOQ:

In UV-Spectroscopy the minimum detectable quantity of an analyte within a sample by an analytical method was determined to be 0.08 µg/ml at 220 nm for LACO and 1.55 µg/ml at 274 nm for IBU, the quantitation limit for a specific analytical method refers to the minimum quantity of the

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substance in a sample that can be accurately and precisely measured which was found to be 0.25 µg/ml at 220 nm for LACO and 4.65 µg/ml at 274 nm for IBU (Table 1). The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed UV spectrophotometric method for the estimation of both drugs. In RP-HPLC the minimum detectable quantity of an analyte within a sample by an analytical method was determined to be 0.02µg/ml for LACO and 0.28µg/ml for IBU at 212 nm, the quantitation limit for a specific analytical method refers to the minimum quantity of the substance in a sample that can be accurately and precisely measured which was found to be 0.07µg/mL for LACO and 0.84 µg/mL for IBU at 212 nm (Table 1). The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed RP-HPLC method for the estimation of both drugs.

4.3.5 Assay as Analysis of Synthetic mixture

From assay, Final concentration of LACO was 2 µg/ml (220 nm) and IBU 16 µg/ml (274 nm) were run into UV and The Percentage assay of LACO and IBU were found to be 99.05% and 99.87 % respectively. Final concentration of LACO was 2 µg/ml and IBU 16 µg/ml at 212 nm were run into RP-HPLC Percentage assay of LACO and IBU were found to be 99.50% and 99.93% respectively. Its results showed in Table 4.

Table 4: Analysis of synthetic mixture for UV Spectrophotometry & RP-HPLC Method

UV-Spectrophotometry Method				
Name of Drug	**Amount in synthetic mixture (µg/ml)**	**Mean Amount found (µg/ml)**	**% Assay ± SD (n=3)**	**%RSD**
Lacosamide oxalate	2	1.981	99.05±0.54	0.548
Ibuprofen sodium	16	15.98	99.87 ± 0.64	0.657
RP-HPLC Method				
Lacosamide oxalate	2	1.99	99.50±0.4	0.436
Ibuprofen sodium	16	15.99	99.93±0.5	0.547

4.3.6 *Robustness:*

Chromatographic analysis was used to analyse the effects of changes in analysts, and the results showed that there was no statistically significant difference in the% RSD. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches' robustness was demonstrated by the %RSD, which remained constant despite minor variations in flow rate, mobile phase composition, and detection wavelength. It was determined that the created approaches were essential as a result.

5. CONCLUSION:

The present work successfully demonstrates the development and validation of first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Lacosamide oxalate and Ibuprofen sodium in a synthetic mixture. Both methods were validated as per ICH $Q_2(R_2)$ guideline and exhibited excellent linearity, accuracy, precision, and sensitivity. The UV method provides a cost-effective and rapid alternative for routine analysis, while the RP-HPLC method offers enhanced specificity and robustness suitable for advanced quality control laboratories. All Developed and Validated Methods were found to be Accurate, Economical, Reproducible and Precise. There was no interference of any Excipients in the determination of Drugs from Synthetic Mixture. So, this Method can be applied for routine Quality Analysis.

ACKNOWLEDGEMENT:

The authors are grateful to Smt. N. M. Padalia Pharmacy College, Ahmedabad, for encouragement and for providing the necessary facilities to carry out this research work.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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