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Eco-Friendly UV/Vis Spectrophotometric Determination of an Anticonvulsant and Cobalamin-Based Supplement in Bulk and Dosage Forms

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Abstract A simple, precise, accurate, and economical UV spectrophotometric method was developed for the simultaneous estimation of lacosamide and cobalamin in bulk and tablet dosage forms. The method was validated as per ICH guidelines for parameters including linearity, accuracy, precision, robustness, and sensitivity. Both drugs followed Beer-Lambert's law in the concentration range of 0.1–0.9 mg/mL for lacosamide and 0.1–0.9 µg/mL for cobalamin, with a correlation coefficient of 0.991. The method showed good sensitivity, with low limits of detection and quantification. The percentage recovery was found to be 99.13–100.2% for lacosamide and 97.46–101.25% for cobalamin, within acceptable limits. Assay results were 100.01% and 100.22% for lacosamide and cobalamin respectively, indicating accuracy. Precision was confirmed by %RSD values below 2%. Robustness was demonstrated by minor changes in NaOH concentration and detection wavelengths. The method showed no interference from excipients and is suitable for routine quality control. Thus, the developed method is a reliable alternative for the simultaneous analysis of lacosamide and cobalamin in combined pharmaceutical formulations.

Keywords: Anticonvulsant, Lacosamide, Cobalamin, UV Spectroscopy, Method Validation

1.INTRODUCTION

The increasing demand for environmentally benign analytical methodologies has driven the advancement of green chemistry practices in pharmaceutical analysis. UV/Visible spectrophotometry, owing to its simplicity, cost-effectiveness, and minimal solvent consumption, stands out as a preferred technique for routine quality control of pharmaceuticals.

Anticonvulsants are a diverse group of drugs primarily used to treat epilepsy and increasingly prescribed for conditions like bipolar disorder and neuropathic pain [1,2]. They work by reducing neuronal excitability, mainly through sodium channel blockade or GABA modulation [3]. Lacosamide, a newer-generation antiepileptic, enhances slow sodium channel inactivation and modulates CRMP-2, making it effective for partial seizures and diabetic neuropathic pain [4,5]. Due to its poor water solubility and lack of official monograph, reliable analytical methods are essential for its estimation. Cobalamin (vitamin B12) is a crucial water-soluble vitamin for neurological and hematological functions, and its analysis also demands sensitive methods due to its complex structure and clinical importance [6,7].

Numerous methods have been reported for the individual estimation of lacosamide and cobalamin using high-performance liquid chromatography (HPLC) [8–11], LC-MS/MS [12], and UV-Visible spectrophotometry [13–15]. For instance, Ganji Ramanaiah et al. [13] developed a sensitive UV-visible spectroscopic method for lacosamide determination with a linear range of 12–40 µg/mL. Similarly, Adosraku and Remmel [15] reported a selective HPLC-UV method for the quantification of cyanocobalamin in pharmaceutical injections with high precision and recovery.

However, simultaneous estimation of both compounds using UV/Vis spectrophotometry remains largely unexplored. UV/Vis spectroscopy is a rapid, cost-effective, and non-destructive technique suitable for the simultaneous analysis of multi-component formulations, especially when the analytes possess overlapping or distinct absorbance maxima. The development and validation of such a method for lacosamide and cobalamin would not only simplify routine analysis in quality control laboratories but also comply with regulatory requirements for method validation as stipulated by the International Council for Harmonisation (ICH) guidelines [16].

Therefore, the present study aims to develop and validate a simple, accurate, and precise UV/Vis spectrophotometric method for the simultaneous determination of lacosamide and cobalamin in bulk and pharmaceutical formulations. The work emphasizes method development, spectral characterization, validation (in terms of linearity, precision, accuracy, and robustness), and application to market formulations, fulfilling a critical gap in analytical methodology for these pharmacologically significant compounds.

1. Material and Method

2.1. Instrumentation

Absorbance measurements were performed using a double-beam PerkinElmer Lambda 25 UV-VIS spectrophotometer with 1-nm spectral bandwidth and ±0.5 nm wavelength accuracy, using 1-cm quartz cells. pH was measured with an Elico pH meter.

2.2. Materials

Lacosamide and Cobalamin (99.9% purity) were procured from Loba Chemie. Analytical-grade reagents and 1.0 M NaOH prepared in double-distilled water were used throughout. 50 mg of each drug was dissolved in 1.0 M NaOH and diluted to 100 mL to obtain 500 µg/mL stock solutions. Aliquots (0.1–0.9 mL) were further diluted to 10 mL.

2.3. Determination of λmax

A 0.5 mg/mL solution of each drug was scanned from **200–800 nm** to determine maximum absorbance wavelengths (λmax), using 1.0 M NaOH as blank.

2. Result and Discussions

3.1. Development and Optimization of Spectrophotometric Method

Method development focused on selecting optimal analytical

conditions based on solubility and spectral behavior of the drugs. Various solvent systems were tested, including water, BR buffer, 1.0 M HCl, and 1.0 M NaOH. Among these, 1.0 M NaOH provided the best solubility and stable absorbance for both lacosamide and cobalamin. Solutions were degassed using an ultrasonic bath before use.

3.2. Selection of Wavelength (λmax)

Standard solutions were scanned from 200–400 nm using 1.0 M NaOH as the blank. The maximum absorbance (λmax) was observed at 217 nm for lacosamide and 265 nm for cobalamin. The absorption spectrum is shown in **Figure 1and 2**

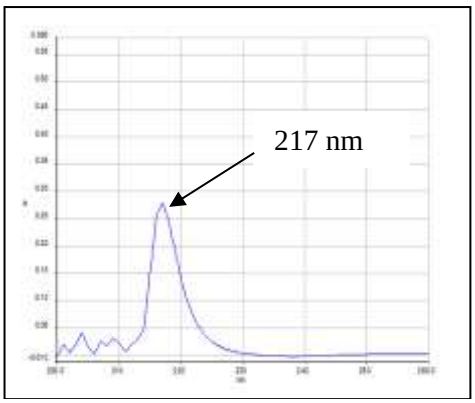

Fig- 1: UV Spectrum of lacosamide

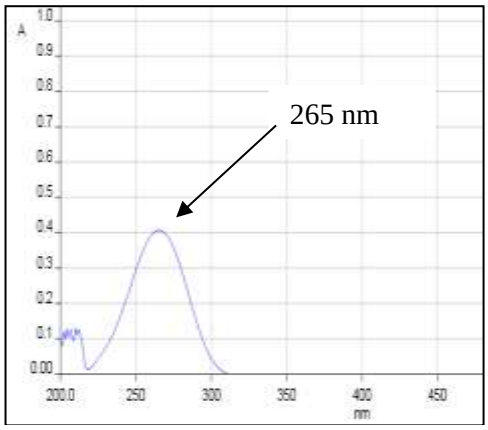

Fig-2: UV Spectrum of cobalamin in NaOH

3.3. Construction of Calibration Curve

A series of standard solutions of Lacosamide (ranging from 0.1 mL to 0.9 mL of the stock solution) were transferred into separate 10 mL volumetric flasks and diluted to volume with 1 M NaOH. The absorbance of each solution was measured at 217 nm for Lacosamide and at 265 nm for Cobalamin. A calibration curve was constructed by plotting the concentration (mg/mL) against the corresponding absorbance values. Both Lacosamide and Cobalamin showed linearity in the concentration range of 0.1 mg/mL to 0.9 mg/mL, as shown in **Figure 3 and 4**

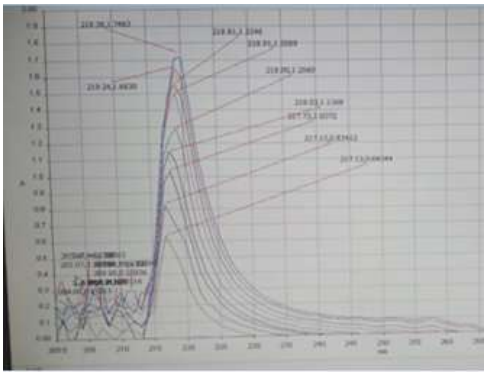

Fig- 3: UV spectrum of Lacosamide in NaOH at different concentration

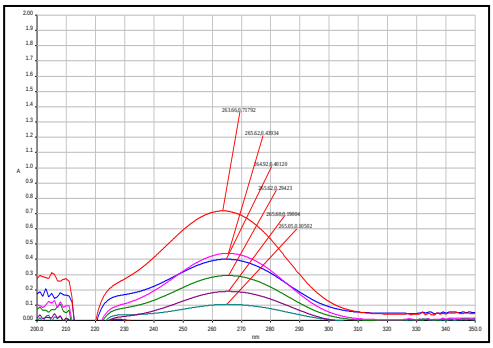

Fig-4: UV spectrum of (Cobalamin in NaOH at different concentration

4.1. Linearity

To establish the linearity of the proposed method, various aliquots of standard solutions of lacosamide and cobalamin were prepared from a stock solution and analyzed in 1.0 M NaOH. Both drugs demonstrated a linear response in the concentration range of 0.1–0.9 mg/mL (Figure 5 and 6). The calibration curve for lacosamide yielded a regression equation of $y = 1.582x + 0.524$ with a correlation coefficient (R^2) of 0.991, indicating excellent linearity. Similarly, cobalamin showed a regression equation of $y = 1.029x – 0.013$ with an R^2 value of 0.996, confirming that the method provides a reliable and consistent response for both analytes across the tested concentration range.

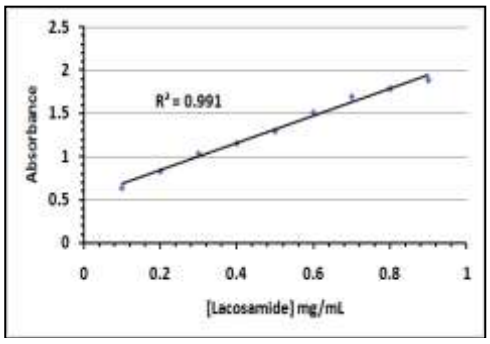

Fig-5: Linearity curve of lacosamide in NaOH

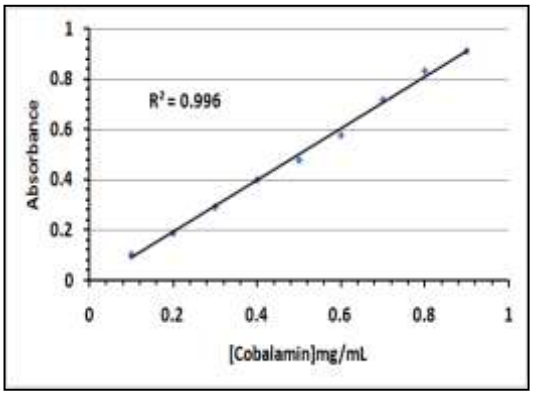

Fig-6: Linearity curve of cobalamin in NaOH

4.2. Accuracy

The accuracy of the proposed method was evaluated through recovery studies by spiking the sample with known amounts of pure lacosamide and cobalamin. The spiked solutions were prepared in triplicate, and the percentage recovery was calculated using the regression equations derived from the calibration curves in 1.0 M NaOH. The results, as presented in **Table 1,** demonstrated good recovery values for both analytes. Lacosamide showed a percentage recovery ranging from **99.13 ± 1.55% to 100.2 ± 3.24%**, while cobalamin exhibited recovery in the range of **97.46 ± 1.39% to 101.25 ± 1.59% (Table 1).** These results confirm the accuracy and reliability of the method for the quantitative estimation of both compounds.

Table-1: Accuracy for lacosamide and cobalamin in NaOH

Lacosamide				Cobalamin			
Amount added (mg/ml)	A	% Recovery	% Recovery ± SD (RDS)	Amount added (mg/ml)	A	% Recovery	% Recovery ± SD (RDS)
0.300	0.9908 0.9897 1.0003	98.357 98.125 100.92	99.134±1.55 (1.56%)	0.300	0.2942 0.3012 0.2898	99.514 101.782 98.089	99.75±1.86 (1.87%)
0.600	1.4919 1.4879 1.4636	101.97 101.54 98.989	100.0±1.61 (1.60%)	0.600	0.5981 0.5812 0.5961	98.980 96.242 97.182	97.46±1.39 (1.43%)
0.900	1.9863 1.8993 1.9698	102.7 96.594 101.54	100.2±3.24 (3.23%)	0.900	0.9121 0.9212 0.9410	99.892 100.87 103.01	101.25±1.59 (1.57%)

4.3. Precision

The precision of the proposed method was evaluated using standard solutions of lacosamide and cobalamin at 0.300 and 0.600 mg/mL in 1.0 M NaOH. Intraday precision was assessed by analyzing six replicates at different time intervals within a single day, while interday precision involved analysis over three consecutive days. The %RSD for lacosamide was 0.70% (intraday) and 0.68% (interday), and for cobalamin, it was 1.12% (intraday) and 1.48% (interday). All values were within the acceptable limit of less than 2%, indicating that the method is precise **(Table 2 and 3).**

4.4. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined to assess the sensitivity of the proposed method. LOD is the lowest concentration of analyte that can be reliably detected, while LOQ is the lowest concentration that can be quantitatively determined with suitable precision and accuracy. These parameters were calculated using the equations proposed by Miller and Miller (1993), where LOD = 3.3(SD/m) and LOQ = 10(SD/m), with SD representing the standard deviation of the y-intercept and *m* the slope of the calibration curve. The LOD and LOQ values for lacosamide were **0.03014 mg/mL and 0.1004 mg/mL,** respectively. For cobalamin, the values were **9.84×10^{-3} µg/mL** (LOD) and **3.28×10^{-2} µg/mL** (LOQ), indicating that the method is highly sensitive for both analytes.

Table- 2: Results of Intraday precision for lacosamide and cobalamin in NaOH

Drug	Drug mg/ml	Replicate No.	Absorbance	Statistical Analysis
Lacosamide	0.600	1	1.4919	SD: 0.0104 Mean: 1.483 % RSD: 0.70
		2	1.4879	
		3	1.4789	
		4	1.4878	
		5	1.4879	
		6	1.4636	
Cobalamin	0.600	1	0.5812	SD: 0.0065 Mean: 0.5839 % RSD: 1.12
		2	0.5971	
		3	0.5812	
		4	0.5798	
		5	0.5812	
		6	0.5830	

Table- 3: Results of Interday precision for lacosamide and cobalamin in NaOH

Drug	Conc (mg/ml)	Absorbance			Average %RSD
		1 day	2 days	3 days	
Lacosamide	0.3	0.989	0.9908	0.9888	0.68%
		0.9997	0.9697	0.9897	
		0.9888	0.9987	0.9943	
		0.9808	0.9808	0.9888	
		0.9887	0.9887	0.9987	
		0.9887	0.9894	0.9959	
	%RSD	0.61%	1.01%	0.42%	
Cobalamin	0.3	0.2942	0.2988	0.2982	1.48%
		0.2912	0.2978	0.3009	
		0.2998	0.2898	0.2968	
		0.2802	0.2901	0.2968	
		0.2999	0.2949	0.2999	
		0.2969	0.2909	0.2979	
	%RSD	2.52%	1.36%	0.56%	

4.5. Robustness

The robustness of the proposed method was evaluated by introducing small deliberate variations in experimental conditions, specifically by altering the concentration of 1.0 M NaOH by ±0.2%. The results showed no significant effect on the absorbance values, indicating that the method remains reliable under slight changes in conditions. These findings confirm the robustness of the developed method. Detailed results are presented in **Table 4.**

Table -4: Robustness of the proposed method in different concentration of NaOH

Sr. No	Absorbance at Different Concentration of NaOH					
	0.8M NaOH		0.9M NaOH		1.0 M NaOH	
	Lacosamide 0.500mg/mL	Cobalamin 0.500mg/mL	Lacosamide 0.500mg/mL	Cobalamin 0.500mg/mL	Lacosamide 0.500mg/mL	Cobalamin 0.500mg/mL
01	1.2969	0.4793	1.2889	0.4783	1.2989	0.4763
02	1.2887	0.4763	1.2987	0.4793	1.2977	0.4773
03	1.2979	0.4993	1.2999	0.4893	1.2899	0.4843
04	1.2969	0.4783	1.2899	0.4773	1.2999	0.4793
05	1.2959	0.4773	1.2979	0.4703	1.2959	0.4775
06	1.2999	0.4783	1.2969	0.4783	1.2919	0.4733
Mean	1.2960	0.4814	1.2953	0.4788	1.2957	0.4779
SD	0.0034	0.0087	0.0047	0.0060	0.0040	0.0036
%RSD	0.30%	1.83%	0.37%	1.27%	0.31%	0.77%

5. Simultaneous Equation Method Development

To develop the simultaneous equation (SE) method, working solutions of lacosamide and cobalamin were scanned in the UV range of 200–400 nm. The overlain spectra revealed that lacosamide exhibited maximum absorbance at **222 nm** (λ_1), while cobalamin showed maximum absorbance at **263 nm** (λ_2). These wavelengths were selected for simultaneous estimation of both drugs. Standard solutions of lacosamide and cobalamin in the concentration range of **0.1–0.6 mg/mL** were prepared in **1.0 M NaOH**, and their absorbance values were recorded at both selected wavelengths. The concentrations of lacosamide (x) and cobalamin (y) in the sample solutions were calculated using the simultaneous equation method based on absorbance values at 222 nm and 263 nm, applying the following formula:

- A_1 = absorbance of the mixture at λ_1 (222 nm)
- A_2 = absorbance of the mixture at λ_2 (263 nm)
- ε_{11} = absorptivity of lacosamide at 222 nm
- ε_{12} = absorptivity of cobalamin at 222 nm
- ε_{21} = absorptivity of lacosamide at 263 nm
- ε_{22} = absorptivity of cobalamin at 263 nm

Then, the simultaneous equations are:

$$A_1 = \varepsilon_{11} \cdot x + \varepsilon_{12} \cdot y$$

$$A_2 = \varepsilon_{21} \cdot x + \varepsilon_{22} \cdot y$$

Solving for **x** (lacosamide) and **y** (cobalamin):

$$x = \frac{A_1 \cdot \varepsilon_{22} - A_2 \cdot \varepsilon_{12}}{\varepsilon_{11} \cdot \varepsilon_{22} - \varepsilon_{12} \cdot \varepsilon_{21}}$$

$$y = \frac{A_2 \cdot \varepsilon_{11} - A_1 \cdot \varepsilon_{21}}{\varepsilon_{11} \cdot \varepsilon_{22} - \varepsilon_{12} \cdot \varepsilon_{21}}$$

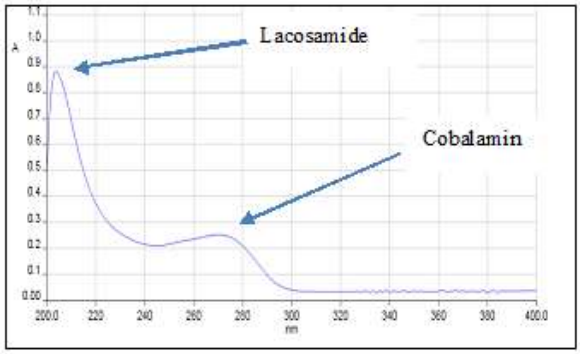

Fig-7: UV spectrum of mixture of lacosamide and cobalamin

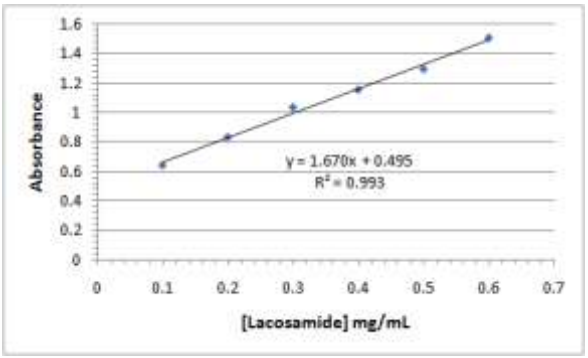

Fig-8: Simultaneous determination graph of lacosamide

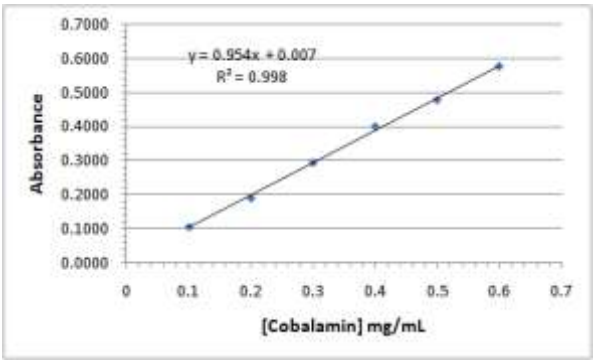

Figure 5: Simultaneous determination graph of cobalamin.

Table -5: Absorptivity value for lacosamide and cobalamin

Concentration mg/mL	Lacosamide		Cobalamin	
	Absorptivity $\lambda 1 - 222nm$	Absorptivity $\lambda 2 - 263$ nm	Absorptivity $\lambda 1 - 222nm$	Absorptivity $\lambda 2 - 263nm$
0.1	449.20	12.1	34.12	253.14
0.2	448.60	12.1	32.12	253.12
0.3	447.30	12.0	34.12	253.15
0.4	449.10	11.8	33.12	254.11
0.5	449.38	12.8	34.12	253.10
0.6	448.95	11.7	33.12	252.98
Mean	448.75	12.08	33.45	253.26
S.D.	±0.7598	±0.3868	±0.8164	±0.4176
RSD in (%)	0.17	3.20	2.44	0.16

5. Application of Developed Method to Marketed Dosage Forms.

The developed UV spectrophotometric method was successfully applied to marketed tablet formulations of lacosamide and cobalamin. The assay results showed good accuracy and precision, with lacosamide and cobalamin contents close to 100% of the labeled claim. The %RSD values were within acceptable limits, confirming the method's suitability for routine quality control analysis **(Table 5).**

Table 5: Assay results of tablets dosage forms.

Lacosamide		Cobalamin	
Amount present (mg)	Amount present (% Label claim)	Amount present (mg)	Amount present (% Label claim)
0.200	101.52	0.19	100.96
0.500	99.036	0.500	99.023
0.700	102.48	0.740	100.70
Mean	101.012	Mean	100.22
S.D.	1.7773	S.D.	1.0513
% RSD	1.76%	%RSD	1.05%

5. Conclusion

A simple and economical UV spectrophotometric method was successfully developed for the simultaneous estimation of lacosamide and cobalamin in combined dosage forms. The method followed ICH guidelines and showed good accuracy, precision, sensitivity, and robustness. Linearity was observed in the range of 0.1–0.9 µg/mL for both drugs with high correlation coefficients. % Recovery values were within the acceptable range, indicating accuracy with no interference from excipients. The % RSD values for intraday and interday studies were less than 2%, confirming good precision. The method showed good LOD and LOQ, proving it is sensitive for low concentration analysis. Robustness was confirmed by varying NaOH concentration and wavelengths without affecting the results. Stability studies showed that the sample solution remained stable for three days at room temperature. The method was successfully applied to marketed tablet formulations, yielding accurate assay results. Thus, this validated method is reliable, simple, and suitable for routine quality control analysis.

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