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Method development and validation for lemborexant by UV spectroscopy

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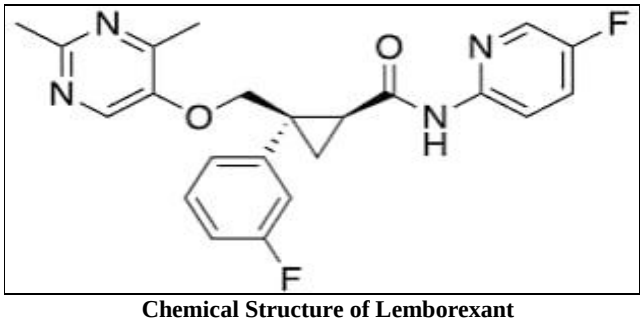
Abstract
A simple, accurate, and precise UV spectrophotometric method was developed and validated to estimate Lemborexant in a marketed formulation. The method showed excellent linearity in the concentration range of 2–14 µg/mL with a strong correlation between absorbance and concentration. The assay of the marketed formulation (Dayvigo, 10 mg) yielded 98.6% of the label claim, confirming method accuracy. Precision studies, both intra-day and inter-day, produced low %RSD values, indicating high reproducibility. Recovery studies demonstrated the method's accuracy with recoveries between 99% and 99.8%. The limit of detection (LOD) and quantification (LOQ) were found to be 6.3 µg/mL and 19.32 µg/mL, respectively. The method also proved robust under slight changes in wavelength, confirming its reliability for routine quality control analysis of Lemborexant formulations.

Keywords: Lemborexant, UV Spectrophotometry, Assay, Validation

Introduction

UV spectroscopy, a widely employed analytical technique, hinges on the principle of measuring the absorption of ultraviolet radiation by a substance. Spectroscopy in general encompasses the generation, measurement, and interpretation of spectra resulting from electromagnetic radiation interacting with matter [1]. Molecular UV-Vis spectroscopy is a versatile technique applicable to a broad spectrum of analytes, with its applications spanning diverse fields such as quantitative analysis, compound identification, and the examination of chemical structures [2]. Lemborexant, marketed as Dayvigo, is a dual orexin receptor antagonist, approved by FDA, is a novel treatment for insomnia characterized by its pyrazolopyridine core with an oxazole ring. Its mechanism of action involves selectively binding to orexin receptors OX1R and OX2R, effectively blocking orexin A and orexin B neuropeptides to reduce wakefulness and promote sleep [3]. Unlike traditional sedatives, Lemborexant does not suppress the central nervous system, offering a potential advantage in terms of safety and side effect profile. This unique combination of structural features and targeted action positions Lemborexant as a promising alternative in the treatment of sleep disorders. The drug's potential benefits, including fewer side effects and improved safety, make it an intriguing subject for further research and clinical application in the field of sleep medicine [4, 5].



Chemical Structure of Lemborexant

Materials and Methods

Equipment

The analytical method was developed and validated using a UV spectrophotometer (LAB INDIA). A Sonicator-ANALAB was also utilized.

Chemicals and reagents: Lemborexant Tablet (Dayvigo), its marketed formulation, and Methanol were used.

Preparation of sample solution [6, 7]

Lemborexant, 10mg, was weighed and transferred to a 10 ml volumetric flask and dissolved in methanol. The content of the flask was sonicated for 20 minutes and made up to the mark with methanol to give 1000µg/ml. From this stock solution, 1ml was pipetted into another 10ml of volumetric flask, and the volume was made up to 10ml with methanol to give 100µg/mL. From this solution, 1 mL was pipetted into another 10 mL volumetric flask, and the volume was made up with methanol to give 10 µg/mL.

Determination of absorption maximum [3, 6]: 1 ml of the standard solution was taken into a 10ml volumetric flask. The mixture was further diluted with methanol to make up to 10ml. The optical absorbance was measured in the wavelength range from 200 to 400nm against a blank of methanol. It shows the maximum absorbance at 287nm. As shown in the figure No.1.

Method Validation [8]

Linearity

The ability of the assay value to be directly proportional to the concentration of an analyte in the sample is called linearity.

Linearity studies were performed by taking a 100µg /ml standard stock solution of Aprelimast and further diluting to obtain 2ug/ml-14µg/ml solutions as shown in Table No.2. The linearity curve was obtained by plotting the

concentration on the X-axis and absorbance on the Y-axis, and a regression equation was calculated as shown in Fig.No.2.

Precision

The system precision is a measure of the method's variability. It was determined by performing three replicate analyses of the same working solutions, the precision method was demonstrated by Intraday and interday variation studies.

The intra precision of the developed UV method was determined by preparing a sample of Lemborexant standard solution at a 2 µg/mL concentration. Measure the absorbance of the 2 µg/mL solution six times consecutively using the same cuvette and solution, as shown in Table no.3. The Interday precision was determined with three concentrations (2,6,10µg /ml) and for three replicates (n=3) samples. Measure and record the absorbance of each of the three concentrations on Day 1, Day 2, and Day 3. The mean, standard deviation, and percentage RSD of the results were used to evaluate the method's precision as shown in Table no.4.

Accuracy

Accuracy of the method was ascertained by the standard addition method at 3 levels. Standard Quantities equivalent to 80%, 100% and 120% are to be added to the sample.

From the standard stock solution of 10µg /ml, take 1 ml into 3 different 10 ml volumetric flasks and label as flask 1, flask 2, and flask 3. To flask 1, add 0.8ml of sample stock solution of 10 µg/mL. to flask 2, add 1ml of sample stock solution of 10µg /mL. To flask 3, add 1.2 mL of sample stock solution and make up the final volume with ethanol to make 80%, 100% and 120% spiking, shown in Table no.5.

LOD and LOQ

Detection limit is the smallest drug quantity that can be detected under normal test conditions. Quantification limit is the lowest drug concentration that can be accurately and precisely determined. LOD and LOQ were determined based on the standard deviation of the response and slope.

Robustness

A measure of a method's capacity to remain unaffected by small, deliberate variations in method parameters, and indicates its reliability during normal usage.

The study was carried out by taking 4µg/ml solution by changing the wavelength at 276nm and 287nm for three replicates (n=3) samples. Measure and record the absorbance, shown in Table no.6.

Results

Table No. 1: Assay analysis of Marketed formulation

Name	Label claim	Amount found	%Estimated	SD*	RSD*
Lemborexant (Dayvigo)	10mg	9.89	98.9	0.02	1.04

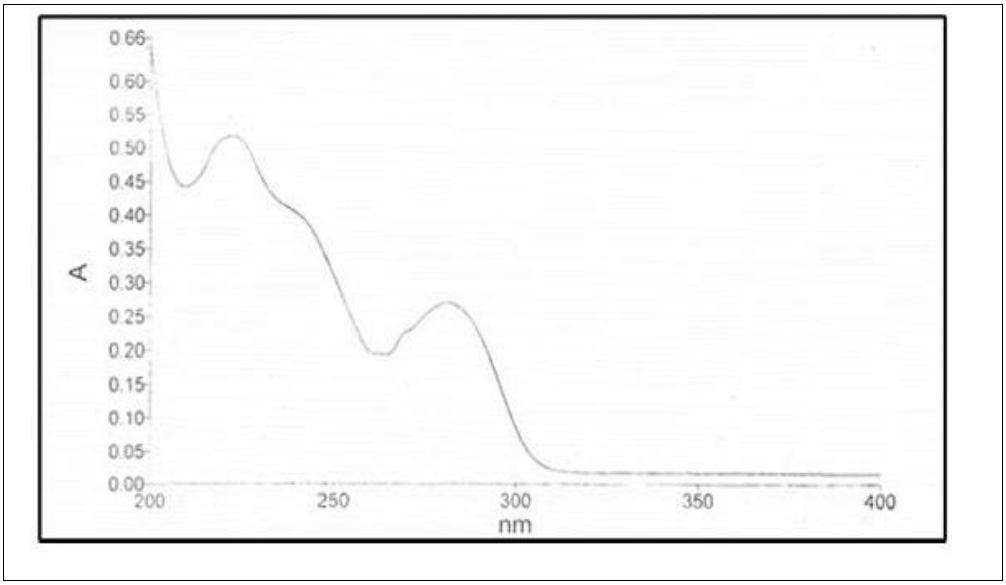


Fig 1: λmax measurement

Linearity

Table 2: Linearity of Marketed formulation

S.NO.	Volume of Solution	Concentration (µg/ml)	Absorbance
1	0.2	2	0.207
2	0.4	4	0.31
3	0.6	6	0.422
4	0.8	8	0.534
5	1	10	0.647
6	1.2	12	0.751
7	1.4	14	0.835

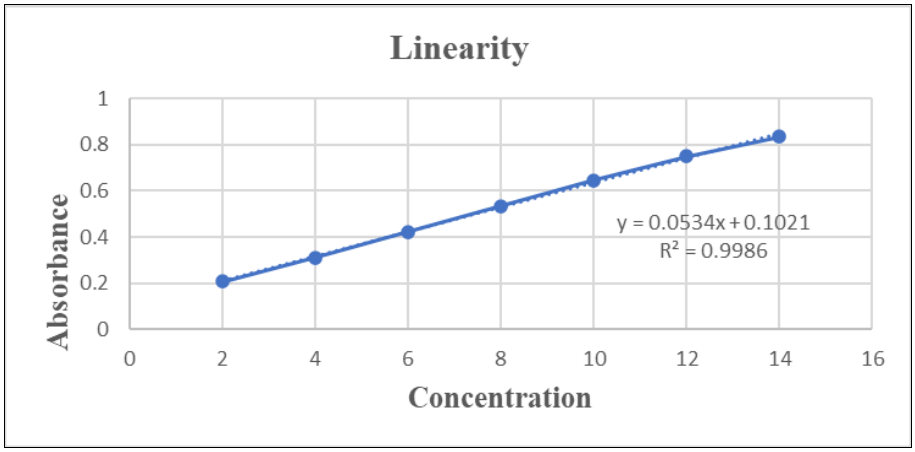


Fig 2: Calibration curve of the Marketed formulation

Precision -Intra Day

Table 3: Precision data of intra-day for Marketed formulation

S.No.	Concentration(µg/ml)	Absorbance	Mean± SD	%RSD
1	2	0.207	0.208±0.002	0.99
2	2	0.208		
3	2	0.212		
4	2	0.211		
5	2	0.208		
6	2	0.207		

Precision -Inter Day

Table 4: Precision data of inter-day for Marketed formulation

S.No.	Concentration (µg/ml)	Absorbance			Mean± SD	%RSD
		Day -1	Day -2	Day -3		
1	2	0.207	0.21	0.209	0.208±0.0015	0.73
2	6	0.422	0.423	0.421	0.422±0.001	0.23
3	10	0.647	0.649	0.667	0.654±0.011	1.6

Accuracy

Table 5: Accuracy data for Marketed formulation

S. No	Levels	Concentration	Amount added	Amount Recovered	% Recovery ± SD
1	80%	100	8	7.92	99±0.5
2	100%	100	10	9.98	99.8±0.5
2	120%	100	12	11.96	99.6±0.5

LOD & LOQ

LOD = 3.3 * SD/S
SD = Standard deviation of slope
S = Slope of calibration
LOD = 3.3* 0.102/0.0534 = 6.3
LOQ = 10 * SD/S
LOQ = 10*0.102/0.0534= 19.32

Robustness

Table 6: Robustness data for Marketed formulation

Drug	Wavelength	
Lemborexant	276	287
	0.542	0.647
	0.544	0.648
	0.543	0.648
Mean	0.543	0.648
Standard deviation	0.001	0.0006
%RSD	0.184	0.089

Discussion

The UV spectrophotometric method developed for the quantitative estimation of the analyte was successfully validated as per ICH guidelines.

The plot showed a straight-line relationship between concentration and absorbance, indicating good linearity over the tested range (2–14 µg/mL). The R^2 value of 0.9986 is very close to 1.000, which confirms a high degree of correlation and reliability of the method for quantitative analysis. The slope (0.0534) and intercept (0.1021) of the line define the sensitivity of the method.

Precision studies, both intra-day and inter-day, demonstrated excellent reproducibility. In the intra-day precision test at a concentration of 2 µg/mL, the absorbance values showed a %RSD of 0.99%, indicating good repeatability. Inter-day precision evaluated over three days at concentrations of 2, 6, and 10 µg/mL yielded %RSD values of 0.73%, 0.23%, and 1.6% respectively, all within acceptable limits, confirming intermediate precision.

Accuracy was assessed through recovery studies at three levels: 80%, 100%, and 120%. The percentage recoveries ranged from 98% to 99% with a standard deviation of ±0.5%, indicating that the method is highly accurate and free from interference by excipients or formulation components.

Sensitivity was evaluated by calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ), which were found to be 6 µg/mL and 19.2 µg/mL, respectively, showing the method is capable of detecting and quantifying low concentrations of the analyte.

Conclusion

The developed UV spectrophotometric method for the estimation of Lemborexant is simple, accurate, precise, and robust. It meets all validation parameters, including linearity, precision, accuracy, sensitivity, and robustness. The low %RSD and high recovery values confirm its reliability and reproducibility. Therefore, this method can be effectively employed for routine quality control analysis of Lemborexant in its marketed formulation.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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