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METHOD DEVELOPMENT AND VALIDATION OF LEMBOREXANT IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM BY REVERSE PHASE–HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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ABSTRACT

A simple, precise, rapid, and reproducible RP -HPLC method was developed and validated for the determination of Lemborexant in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a Kromasil-18 ;(ODS) column (250 X 4.6 mm i.d., particle size 3.5µ).The mobile phase consisted of Pentane sulphonic acid sodium salt mono hydrate, add 1ml of Perchloric acid and adjust the pH-2.7±0.05 with Triethyl amine: Methanol in the ratio 40: 60 v/v. An isocratic elution at a flow rate of 1.0 ml/ min at ambient temperature. The detection was carried out at 237 nm using waters UV-Visible detector. The calibration curve was linear in the concentration range of 2–12µg/ ml (r2= 0.9997).The limit of detection and the limit of quantification were found to be 0.273 µg/ml and 0.676 µg/ml respectively. The amount of Lemborexant present in the formulation was found to be 99.95. The method was validated statistically using the SD, %RSD and SE and the values are found to be within the limits and the recovery studies were performed and the percentage recoveries was found to be 99.55± 0.7211 %. So, the proposed method was found to be simple, specific, linear, and rugged. Hence it can be used for applied for routine analysis of Lemborexant in the Pharmaceutical formulations.

KEYWORDS: Lemborexant**,** RP-HPLC, UV detection, Development and validation of method; Tablet dosage form.

INTRODUCTION

Lemborexant, sold under the brand name Dayvigo, is a medication for the treatment of insomnia characterized by difficulties with sleep onset and/or sleep maintenance in adults.[1-4] Lemborexant was approved in the United States for use by adults with insomnia in December 2019. Lemborexant is chemically (DAYVIGO) contains lemborexant, an orexin receptor antagonist. The chemical name of lemborexant is (1R,2S)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)-N-(5-fluoropyridin-2-yl) cyclopropanecarboxamide. The molecular formula is $C_{22}H_{20}F_2N_4O_2$. The molecular weight is 410.42. The structural formula is:. Lemborexant is also having maximum half-life in sartans - 24 hrs. (Fig.1). LEMBOREXANT is not official in any pharmacopoeia.

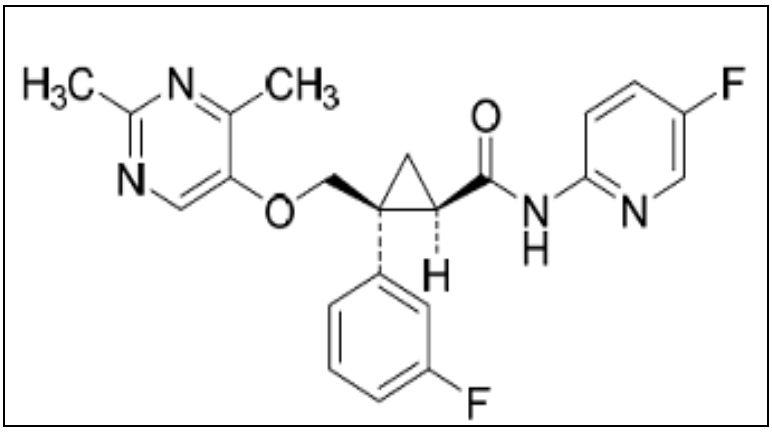

Fig. 1: Chemical structure of lemborexant.

Lemborexant is a white to off-white powder that is practically insoluble in water. DAYVIGO tablets are intended for oral administration. Each film coated tablet contains 5 mg or 10 mg of Lemborexant. The inactive ingredients are: hydroxypropyl cellulose, lactose monohydrate, low-substituted hydroxypropyl cellulose, and magnesium stearate.

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of Lemborexant in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 5 min) Lemborexant, good precision (R.S.D.less than 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH) for the determination of Lemborexant in bulk and in tablet dosage form.

Experimental

Instrumentation

Quantitative HPLC was performed on a gradient High Pressure Liquid Chromatography (waters separation module) with variable wave length programmable Diode array Detector 2487, and Kromasil-18 Column. The HPLC system was equipped with the software "Empower-2 series (waters)".

Preparation of standard drug solution

Stock solutions (0.48 mg/ml) of Lemborexant was prepared by dissolving 48mg of Lemborexant in 100 ml volumetric flasks add about 75ml of diluent, add 2ml of 1N NaOH solution and sonicated for about 15 min. and then made up to volume with diluent. Dilute 5ml of this solution to 100ml with diluent. Filter the solution through 0.45µm nylon filter. The stock contains 6µg/ml.

Preparation of sample drug solution for pharmaceutical formulations

Ten tablets were taken and pulverized. The sample of the powdered tablets were transferd into a 1000ml volumetric flask and add about 750ml of diluent, add 20 ml of 1N NaOH solution and sonicate for about 45 min with intermittent shaking to disperse the tablets completely and makeup with diluent. Dilute 3ml of this solution to 100ml with diluent and filter through 0.45µm nylon filter; finally we get the concentration obtained was 24µg/ml After systematic and detailed study of the various parameters involved, as described under results and discussion in this chapter, the following procedure was recommended for the determination of Lemborexant in bulk samples and pharmaceutical formulations.

Preparation of buffer

Weigh and dissolve about 1g of Pentane sulphonic acid sodium salt mono hydrate in 1000ml water, add 1ml of Perchloric acid and adjust the pH-2.7±0.07 with Triethyl amine. Filter the solution through 0.45 µm membrane filter.

Preparation of mobile phase

The mobile phase was prepared by mixing the buffer and methanol in the ratio of 40:60 v/v and the solution was filtered and degassed.

Preparation of diluent

The diluent was prepared by mixing water and methanol in the ratio of 60:40 v/v and the solution was filtered and degassed.

Chromatographic conditions

Column	:	Kromasil-18, 150 mm x 4.6 mm; 3.5µ
Flow rate	:	1.0 ml/min
Run time	:	10 min
Column temperature	:	27±1 °c
Injection volume	:	10 µl
Detection wavelength	:	237nm

3. RESULT AND DISCUSSION

3.1 Method development

The method utilizing Methanol: Water as mobile phase yielded broad peak, whereas with MeOH: Water tailing was observed with methanol as diluent. Procedure utilizing Methanol: Water as mobile phase with water as diluents also yielded tailing where as with MeOH: Water mobile phase and acetonitrile as diluent sharp peak was obtained. During method development, a number of variations were tested like Methanol concentration and flow rate to give a symmetric peak. With a mobile phase Methanol: Water (75:25) at flow rate 1 ml min-1 and wavelength is 237 nm, symmetric peak was obtained [Fig. 2].

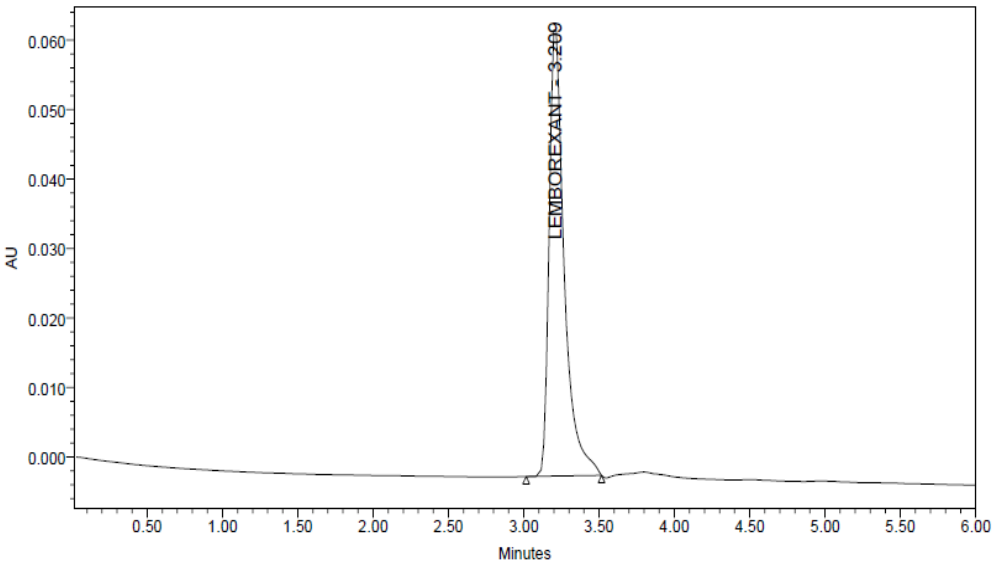

Fig. 2.0 Typical chromatogram for lemborexant.

3.2 Validation

3.2.1 Linearity

Six serial dilutions were prepared in concentration range from 2 to 12 µg/ml. A volume of 10 µl from each concentration of the solution was injected and chromatograms were recorded; three independent determinations were performed at each concentration. A linear calibration graph (y = 2086x + 7194; where y and x are peak area and concentration, respectively) was obtained over six concentrations 2, 4, 6, 8, 10 and 12 µg/ml. Correlation coefficient was found to be 0.999.

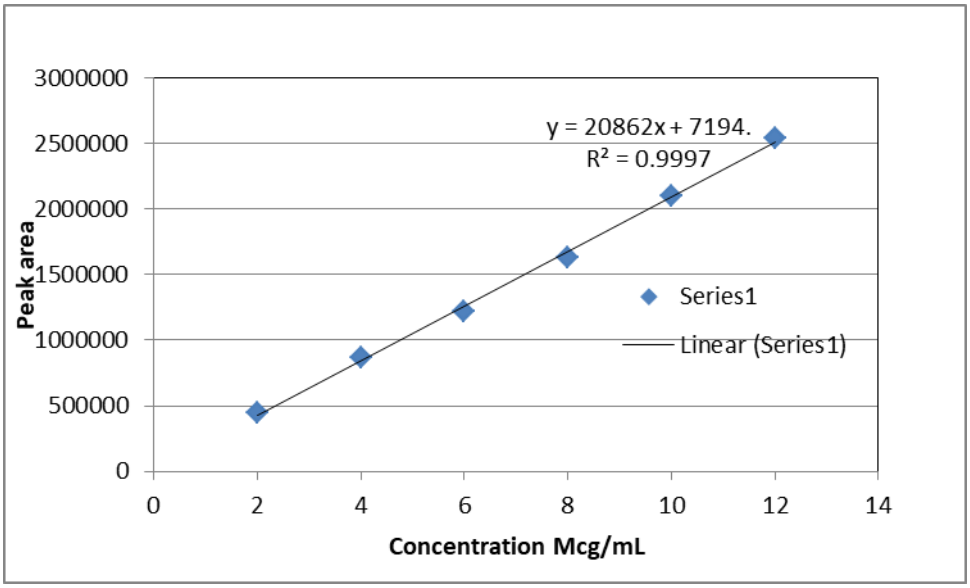

Fig. 3.0. Calibration curve of lemborexant.

Table 01: Optical characteristic of lemborexant.

Parameter	Lemborexant
Retention time (t) (min)	3.2
Theoretical plates (n)	8375
Plates per meter (N)	55793
(HETP)(mts)	1.70×10^{-4}
Tailing factor (T)	1.10
Linearity range (µg ml^{-1})	2-12
Regression equation (Y = a + bC)	0.9997
Correlation coefficient (r)	0.999
Relative standard deviation (%)*	0.127

3.2.2 Accuracy

To ensure the accuracy of the analytical method, the recovery studies were carried out. Known amount of Lemborexant was added to a pre quantified sample solution of its dosage form and the amounts of Lemborexant were estimated by measuring the peak area ratios and

by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range of Lemborexant. Accuracy was evaluated at three different concentrations equivalent to 50% to 150% (50%, 100% & 150%) of the active ingredient by calculating the recovery of Lemborexant with %RSD.

Table 02: Accuracy of lemborexant.

Sample No.	Spike level	µg/ml added	µg/ml found	% Recovery	Mean% Recovery
1.	50	5	4.9	99.75	99.19
2.	50	5	4.8	98	
3.	50	5	4.9	99.75	
1.	100	10	9.9	99.35	99.17
2.	100	10	9.87	99	
3.	100	10	9.9	99.12	
1.	150	15	14.9	99.58	99.47
2.	150	15	14.9	99.25	
3.	150	15	14.8	99.5	

3.2.3 Precision

Intra-day precision of the method was determined by repeat analysis (three identical injections) at three concentration levels. Inter-day precision was established by performing the analysis next day on a freshly prepared solution. The low RSD values of Table 3 indicate the ruggedness of the method. The low RSD values indicate the ruggedness of the method.

Table 3: Precision study.

No. of Injection	% Assay*
1	99.47
2	98.27
3	100.25
4	99.66
5	99.86
6	98.11
Mean	98.79 ± 0.8075
SD	0.4055
%RSD	3032

*n=6

3.2.4 Repeatability

The peak area of 16 ppm drug solution was analyzed six times on the same day. The %RSD was calculated for the resultant peak area.

Table 4.0: Repeatability of lemborexant.

S. No	%Assay
1.	100.1
2.	100.5
3.	100.8
4.	100.1
5.	100.5
6.	99.8
Mean	100.3
%RSD (Limit NMT 2.0%)	0.36

3.2.5 Robustness

The robustness was assessed by altering the following experimental conditions such as, by changing the flow rate from 0.8 to 1.0 ml/min, the mobile phase composition with Methanol: Water (60:43, 60:45) and analyzed in triplicate. In all varied Chromatographic conditions, there was no significant change in chromatographic parameters. There was no effect of mobile phase Composition on retention time as seen in Table.5

Table 5: Robustness data.

System Suitability Parameters	Observed value with Flow rate			Acceptance Criteria
	0.8 ml/min	1.0 ml/min	1.2 ml/min	
Tailing factor for Lemborexant peak	1.043	1.042	1.041	NMT 2.0
Relative standard deviation of Lemborexant pea k area for five injections of standard.	0.08%	0.05%	0.03%	NMT 2.0%

3.2.6. Ruggedness

The % assay and RSD for samples prepared by second analyst was calculated and found within limit. Then RSD of analyst 1 and analyst 2 was calculated and found within limit. This proved that the method is rugged, as depicted in Table 6.

Table no. 6.0: Ruggedness analysis (*n=6).

Analyst 1 Sample	% Assay	Analyst 2 Sample	% Assay
1	100.04	1	99.85
2	100.22	2	98.91
3	99.84	3	100.45
4	100.35	4	100.03
5	100.67	5	99.47
6	100.41	6	99.49
*Mean	100.25	Mean	99.75
SD	0.283	SD	0.193
RSD	0.271	RSD	0.159

CONCLUSION

A RP-HPLC method has been developed for the determination of Lemborexant. The proposed method is simple, rapid, accurate and precise. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Lemborexant. The results of the study reveal that the proposed RP-HPLC method for the estimation of Lemborexant is simple and accurate in bulk and pharmaceutical dosage forms.

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REFERENCES

1. Available from : http//www.en.wikipedia.org/wiki/ Lemborexant
2. Available from: http//www.pubmed.pharmacol.com/ Lemborexant.
3. Available from: http//www.Rx list.com/ Lemborexant
4. Sethi, P.D. HPLC Quantitative Analysis of Pharmaceutical Formulations. CBS publisher and Distributors, New Delhi, 2001; 1: 5-10.
5. Llyod, R., Synder., Joseph, K., Kirkl., Joseph., and L. Glajch. Practical HPLC Method Development. Wiley –Interscience Publication, New york, 1997; 2: 45.
6. Anonymous. Shimadzu LC-10 ATVP High performance liquid chromatography Instruction Manual, Shimadzu Corporation., Kyoto, Japan, 2001; 11-2.
7. Shin JM, Choo YM, Sachs G. Aliment Pharmacol Ther., Blackwell Publishing Ltd, 2006; 2-8.

8. Sharma, B.K. Instrumental Methods of Chemicals Analysis. Goel Publisher House, Meerut, 1994; 13: 7.
9. Mendham, J., Denny, R.C., Barnes, J.D. and Thomas, M.J.K. Vogel's, Text book of Quantitative Chemical Analysis. Pearson Education Pvt. Ltd., New Delhi, 2002; 6: 261-263, 268,277,653,654.
10. James, W. and Munson. Pharmaceutical Analysis Modern Methods Part B. International Medical Book Distributors, Mumbai, 2001; 16: 51-76.
11. Code Q2A, Text on Validation of Analytical Procedures. ICH Tripartite Guidelines, Geneva, Switzerland, 1994; 27: 1 - 5.
12. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Tripartite Guidelines, Geneva, Switzerland, and 6th November, 1996; 1 - 8.