GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Development and validation of novel HPLC method for analytical evaluation of Lemborexant in tablet dosage form

Siddhesh Nivrutti Kamble [1,*], Vijay Kumar Munipalli [2], Harshada Talapadatur [2], Raman Mohan Singh [2], Sayali Warde [2], Smita Nayak [1] and Bhaskar Vaidhun [1]

[1] *Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai, Maharashtra, India.*
[2] *Central Drug Testing Laboratory, Zonal FDA Bhavan, Mumbai Central, Mumbai.*

GSC Advanced Research and Reviews, 2022, 11(01), 132–143

Publication history: Received on 19 February 2022; revised on 05 April 2022; accepted on 07 April 2022

Article DOI: https://doi.org/10.30574/gscarr.2022.11.1.0086

Abstract

A simple and novel isocratic high-performance liquid chromatography (HPLC) method was developed for quantitative determination of lemborexant in bulk as well as in its tablet dosage form. The developed HPLC method was validated as per ICH (Q2R1) guideline. The results of validation parameters indicate that developed HPLC method was specific, accurate, precise, rapid, reliable and reproducible. Therefore, it can be applied for routine quality control analysis of Lemborexant in bulk and tablet dosage form. Materials and Methods**:** The chromatographic separation was achieved on Thermoscientific BDS Hypersil (C18, 15 cm × 4.6 mm id, 5µ) column. The mobile phase consisting of buffer (triethylamine (0.1% v/v) adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile in the ratio of (60:40) was passed through the column maintained at 40 °C with a flow rate of 1 ml/min. Approximately 20 µl of the solution was injected and the analyte was eluted at 265 nm.

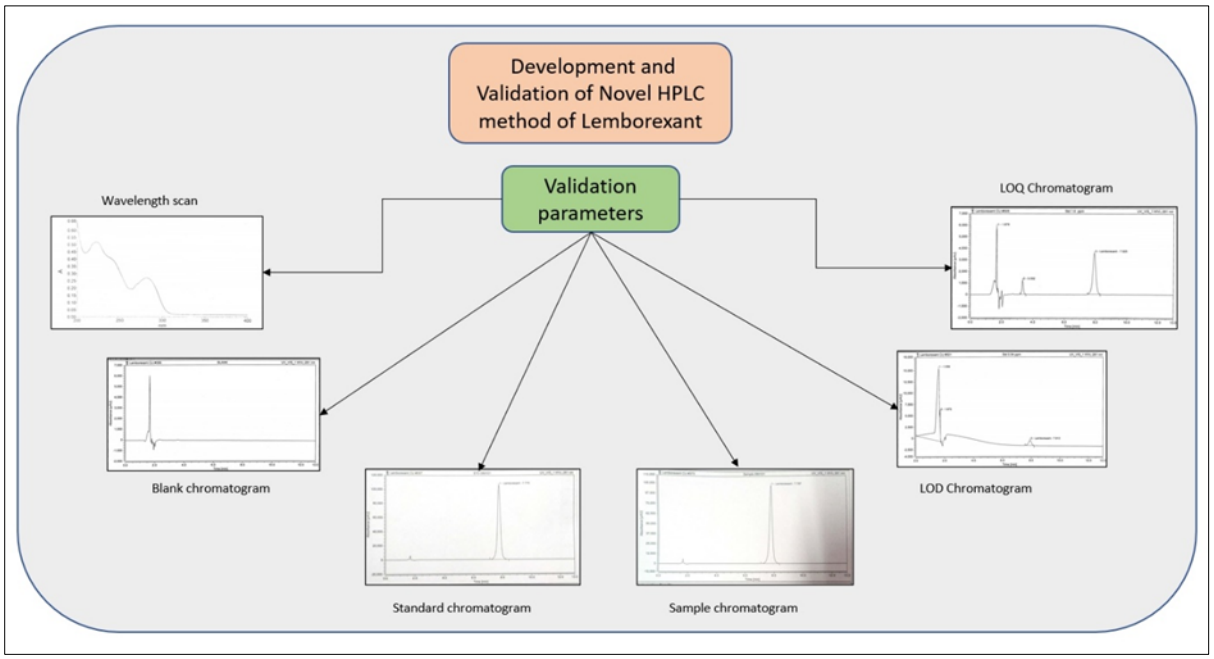
Results: The retention time of lemborexant was around 7.77 min. The percentage RSD of each parameter was found within the limit. The recovery of lemborexant was found to be 100.71%. LOD and LOQ values of lemborexant were found to be 0.54 µg/ml and 1.6 µg/ml respectively. The method was linear over the range of 10-70 µg/ml with a regression coefficient 0.9996. All the verification parameters were within the range according to ICH guidelines.

Conclusion: The developed RP-HPLC method is economical, simple, and practical and useful in routine analysis of Lemborexant in bulk and tablet dosage form.

Keywords: Lemborexant; RP-HPLC; Method development; Validation; Quantitative determination

Graphical abstract

* Corresponding author: Siddhesh Nivrutti Kamble; Email: siddheshkamble98@gmail.com
Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai, Maharashtra, India.



1. Introduction

Lemborexant is an orally administered dual orexin receptor antagonist used in the treatment of insomnia characterized by difficulties with sleep onset and sleep maintenance in adults. It is sold under the brand name Dayvigo™. Orexins are neuropeptides which regulate the sleep-wake cycle and help to promote wakefulness by binding to the G-protein–coupled receptors, OX1R and OX2R.[1-3] They interfere with orexin neurotransmission to facilitate sleep onset and maintenance without interfering with ability to awaken to external stimuli. Lemborexant was approved by the US FDA in 2019 for the treatment of adult patients with insomnia. Later it was also approved by Japan in 2020 for treatment of insomnia.[4-7] The chemical name of Lemborexant is (1*R*,2*S*)-2-[(2,4-dimethylpyrimidin-5-yl) oxymethyl]-2-(3-fluorophenyl)-*N*-(5-fluoropyridin-2-yl) cyclopropane-1-carboxamide (Figure 1). The molecular formula is $C_{22}H_{20}F_2N_4O_2$. The molecular weight is 410.4 g/mol.[8-10.]

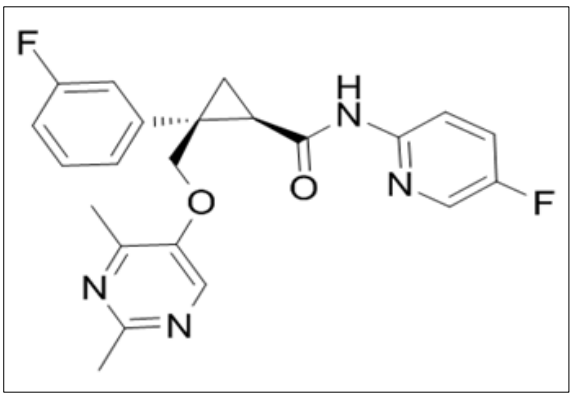


Figure 1 Structure of Lemborexant

Survey of literature revealed one RP-HPLC method that was developed for the determination of lemborexant in bulk and pharmaceutical dosage form using UV detector. Separation was carried out on Kromasil-18 ;(ODS) column (250 X 4.6 mm i.d., particle size 3.5µ). The mobile phase included pentane sulphonic acid sodium salt monohydrate, 0.1% v/v of perchloric acid and pH adjusted to 2.7± 0.05 with triethylamine: methanol in the ratio 40: 60 v/v. The flow rate was adjusted to 1.0 ml/ min at ambient temperature. The detection was carried out at 237 nm using Waters UV-Visible detector. Linearity was observed in the range of 2–12 µg/ ml (r^2= 0.9997). The method was validated statistically by determining SD, % RSD and SE and the values were found to be within limits.[11] In the reported method, the peak shows tailing and asymmetry is high. Hence trials were planned to develop more precise, reproducible, reliable and accurate method that complies with the necessary requirements.

2. Material and methods

2.1. Chemicals and reagents

Lemborexant with defined potency was procured from Central Drugs Testing Laboratory, Mumbai. DAYVIGO™ (10mg) Tablet were also procured from Central Drugs Testing Laboratory, Mumbai. Acetonitrile (HPLC grade) from Merck Life Science, methanol (HPLC grade) from Molychem, triethylamine from Rankem, orthophosphoric acid was from Avra. Ultrapurified HPLC grade water was obtained from the Milli - Q® system (Millipore, Milford, MA, USA) water purification unit. Mobile phase was filtered using 0.45µ nylon filters by Millipore (USA) and was sonicated and degassed using sonicator.

2.2. Instrumentation

Perkin Elmer UV/Vis Spectrometer Lambda 25 connected to a computer loaded with software was used in spectrophotometric measurements. Perkin Elmer UV Win Lab software platform was used for data collection, processing and generating results. Chromatographic evaluation was performed on Thermo scientific Dionic Ultimate 3000 HPLC using software Chromeleon 7. Eutech pH meter was used to maintain the ionic concentration. Sartorius Analytical Balance was used for all the weighing.

2.3. Selection of detection wavelength of Lemborexant

Accurately about 10 mg of API was transferred to the 100 ml volumetric flask and the volume was made up to the mark with diluent (100 µg/ml). The aliquot portion of standard stock solution of lemborexant was diluted appropriately with diluent to obtain concentration of 10.0 µg/ml. The solutions were scanned in the range of 200 to 400 nm. Lemborexant showed maximum absorbance at 281 nm as shown in Figure 2. So, the wavelength selected for the HPLC analysis of lemborexant was 281 nm.

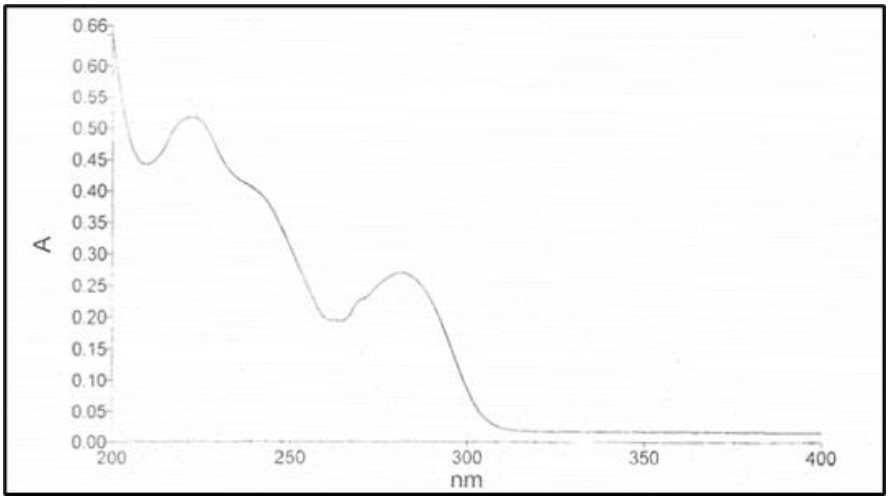


Figure 2 Determination of detection wavelength of Lemborexant

2.4. Preparation of mobile phase

Mobile phase consists of buffer (triethylamine (0.1% v/v) adjusted to pH 3.0 with Orthophosphoric acid) and acetonitrile in the ratio of (60:40) at 40 °C. Two different ports were used for running of mobile phase in isocratic form. To prepare 0.1 % v/v triethylamine 1 ml of triethylamine was dissolved into 1000 ml of HPLC grade water and pH adjusted to 3 using Orthophosphoric acid. The mobile phase was vacuum filtered through 0.45µm high flow nylon membrane filters purchased from Axiva Sichem Pvt. Ltd and was sonicated and degassed using ultra sonicator.

2.5. Preparation of Standard solution

Lemborexant (10 mg) was transferred to a 100 ml volumetric flask and dissolved in 50 ml of diluent. The contents of the flask were sonicated for 10 min and made up to the mark with diluent to obtain a standard stock solution having a concentration of 100 µg/ml. This solution was further diluted to achieve a concentration of 50 µg/ml.

2.6. Preparation of Sample solution

Twenty tablets (DAYVIGO™ Tablets 10 mg) were weighed and average weight was calculated. The tablet contents were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 10 mg of lemborexant was dissolved in diluent and sonicated for 15 min. Volume was made up to 100 ml with diluent (100 µg/ml) and final dilution was done to get a concentration of 50 µg/ml.

3. Validation of method

The developed RP-HPLC method of Lemborexant was validated as per ICH Guidelines Q2 (R1) for parameters such as system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection and limit of quantification.[12]

3.1. System Suitability

System suitability testing is an integral part of method development and it was carried out as per ICH Q2(R1) guidelines. A blank preparation (single injection) and a working standard solution (five replicate injections) of concentration of 50 µg/ml were injected into the HPLC and the chromatograms were recorded to evaluate the SST parameters like % RSD of Retention time, Tailing factor and Theoretical plates.

3.2. Specificity

Specificity is the ability to assess equivocally the analyte in the presence of components which may be expected to be present. Specificity study is performed by injecting blank, standard drug (50 µg/ml) and test solution (50 µg/ml) of lemborexant into the HPLC system. Chromatograms shown in Figure 3, 4 and 5 show that there is no interference of excipient peak at the retention time of lemborexant peak.

3.3. Linearity and Range

Linearity of method was studied in the concentration range of 10-70 µg/ml for lemborexant. Concentration of drug on X-axis and the corresponding peak area on Y-axis was used to plot the linearity graph.

3.4. Accuracy (Standard Addition method)

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 estimated value. The accuracy method was established by standard addition method at four different level 100%, 110%, 120% and 130%. At each level, three determinations were performed, the amount recovered, % recovery, and % RSD were taken into consideration.

3.4.1. Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample.

3.4.2. System precision

This was performed by injecting five replicate injections of a standard solution (50µg/ml). The average SD, %RSD of an area in five replicate injections was calculated and reported.

3.4.3. Method Precision (Assay Repeatability)

This was performed by injecting five replicate injections of a standard solution (50µg/ml) and five sample preparations of lemborexant (50µg/ml) in triplicate into the HPLC system. The % assay, average, SD, %RSD were calculated and reported.

3.4.4. Intermediate Precision

This was performed on two different days and two different HPLC instruments. Five replicates of standard solution (50 µg/ml) and one sample preparation (50 µg/ml) in triplicate were injected into the HPLC system. Its percent assay was calculated and reported.

3.5. Robustness

It is assessed by determining the extent to which small or deliberate changes in the experimental parameters affect the analytical results. This was performed by a change in flow rate (± 0.2 ml/min), change in column oven temperature (±3°C), change in wavelength (±2 nm) and change in mobile phase ratio (±2). Three sample preparations of 50 µg/ml were prepared and injected in triplicate along with five replicate injections of a standard solution of 50 µg/ml under different chromatographic conditions. Its % assay, average, SD, % RSD were calculated and reported.

3.6. LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) of lemborexant were determined from the calibration curve method using the following formulas:

$$\mathrm{LOD} = 3.3 \times \alpha/s\,, \mathrm{LOQ} = 10 \times \alpha/s$$

Where α is the standard deviation of the response of the regression line and s is the slope obtained from the calibration curve. The values of LOD and LOQ were calculated and solutions of these concentration were prepared and injected.

3.7. Assay

The optimized method was applied on tablets having a label claim of lemborexant 10 mg. The assay was performed by injecting five replicate injections of standard preparation 50 µg/ml and five sample preparations 50 µg/ml in triplicate were injected into the HPLC system. Its % assay, average, SD, % RSD were calculated and reported.

4. Results and discussion

4.1. Method Optimization

Molecular structure and solubility data shows that, lemborexant is basic non-polar compound. BDS Hypersil column was selected for the better retention of lemborexant. Different compositions of mobile phase solvents were used. Initially the trials were conducted with mobile phase consisting of acetonitrile and water in the ratio of 50:50 v/v % with a flow rate of 1.0 ml/min. But low retention time was observed. Further changes were made in mobile phase consisting of buffer (triethylamine (0.1% v/v) adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile in the ratio of (55:45). The results show slight increase in the retention time and symmetry of the peak. Later change in the ratio of mobile phase to (60:40) was done. Better symmetrical peak shape with acceptable system suitability testing parameters was found. The selected mobile phase and column was then preceded to method validation as per ICH guidelines.

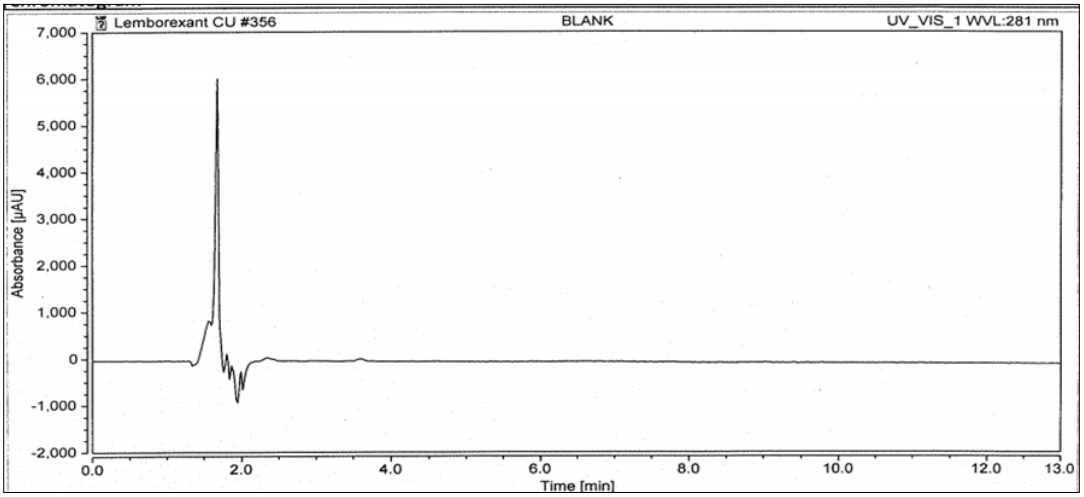


Figure 3 Chromatogram of blank solution

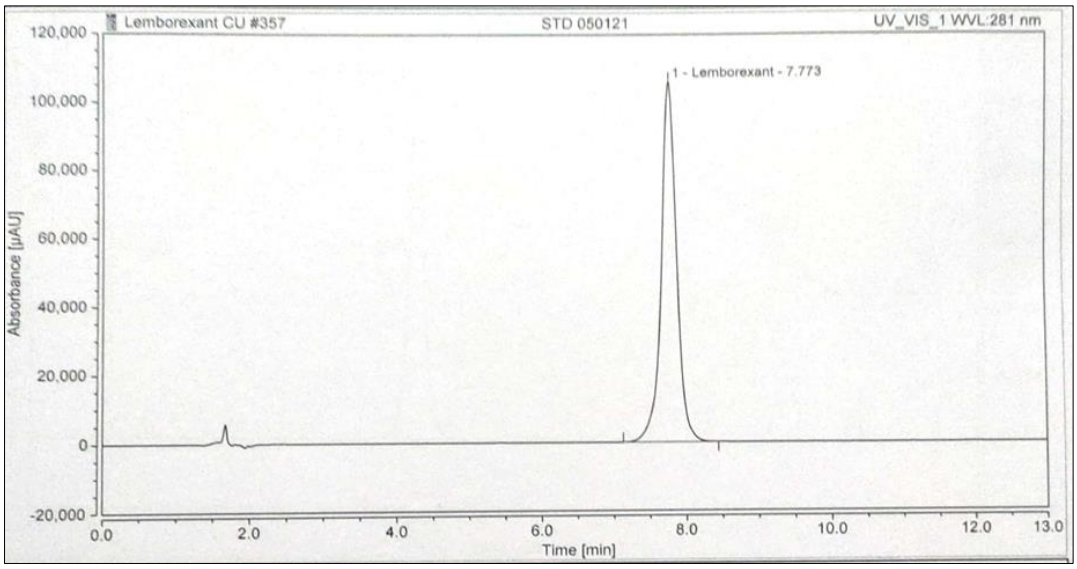


Figure 4 Chromatogram of standard solution

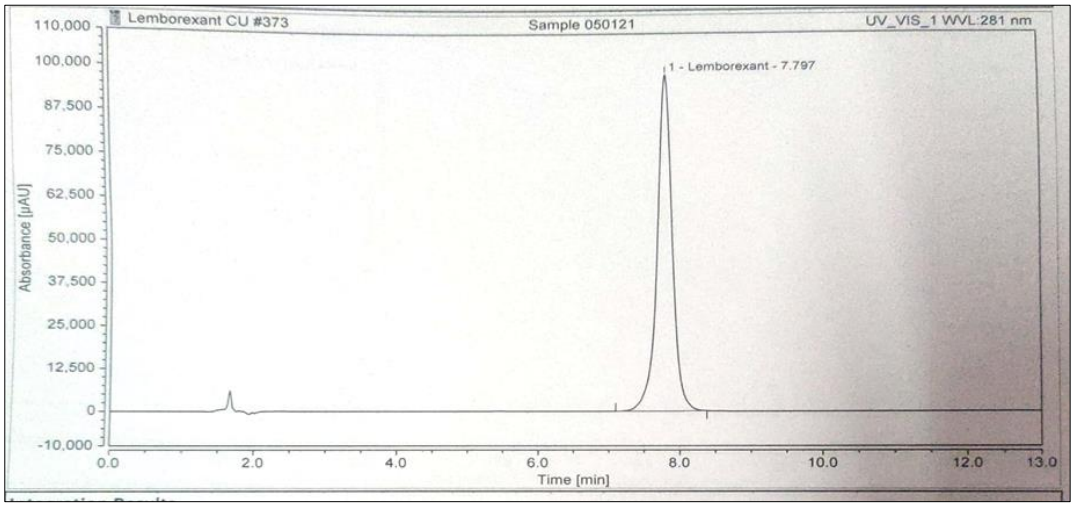


Figure 5 Chromatogram of test solution

4.2. Chromatographic conditions

Column: Thermoscientific BDS Hypersil (C18, 15cm × 4.6mm id, 5µ)
Flow rate: 1 ml/min.
Column Temperature: 40°C.
Autosampler Temperature: 10°C ± 5.
Programming: Isocratic.
Wavelength: 281 nm.
Run time: 13 min.
Injection volume: 20 µl.
Diluent: Methanol: Water (60: 40).

4.3. System Suitability

Before starting sample analysis, the chromatographic system used for analysis must pass the SST limits. All the SST parameters like tailing factor should be less than 2, theoretical plates greater than 6000, and % RSD of peak areas less than 2. In the current method, all parameters were established within the limit which demonstrates that the values are reproducible. The results of system suitability studies are summarized in Table 1.

4.4. Linearity

Linearity was determined over the range of (10-70 µg/ml) for lemborexant. Regression equation obtained was y = 30583.5x - 2162.5 as shown in Figure 6. The method is having good linearity (r^2 = 0.99961). The linearity data is summarized in Table 2.

Table 1 Results of system suitability and system precision parameters of Lemborexant

Sr No	Peak Area	Retention Time (min)	Theoretical plates	Tailing Factor
1	1536138	7.78	20016	1.29
2	1537046	7.73	19709	1.27
3	1533513	7.74	19723	1.27
4	1533594	7.75	19773	1.28
5	1531792	7.76	19710	1.27
Average	1534417	7.7496	19786.2	1.276
S.D.	2136.507	0.0187163	----	0.01
% RSD	0.14	0.24	----	0.7
Limit	N.M.T 2.0%	N.M.T 1.0%	N.L.T. 2000	N.M.T 2.0%

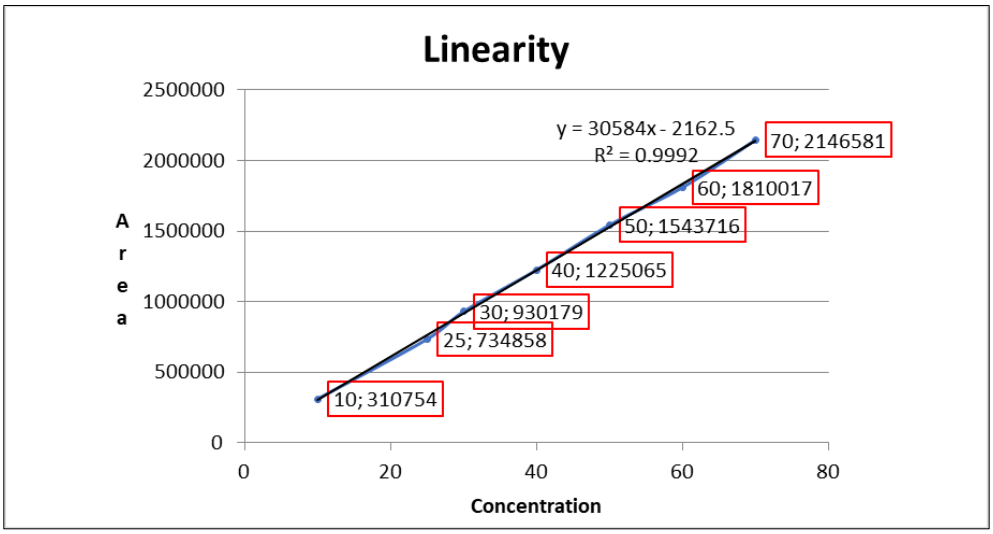


Figure 6 Calibration curve of Lemborexant

Table 2 Linearity data of Lemborexant

Linearity level	Concentration (µg/ml)	Area
1	10	310754
2	25	734858
3	30	930179
4	40	1225065
5	50	1543716
6	60	1810017
7	70	2146581

4.5. Precision

4.5.1. System precision

The % RSD values were found to be within the limit that is less than 2%. The results are summarized in Table 1.

4.5.2. Method Precision

The mean assay percentage results are summarized in the Table No. 3 and are found to be within limit.

4.5.3. Intermediate Precision

The % assay for day-1 and day-2 and HPLC-1 and HPLC-2 were found. The results are summarized in Table 4.

4.6. Accuracy and Recovery

Accuracy results at various levels of concentration are summarized in Table 5. For accuracy studies the limit for percent mean recovery is 98%-102%. From the results, it can be seen that the percent mean recovery is 100.71% which is within the limit, hence the method is accurate.

4.7. LOD and LOQ

The present method can detect and quantify the analyte at lower concentration. Values were estimated as following $\propto = 1229.472$, s =30583.5, LOD = 0.54 µg/ml, LOQ = 1.6 µg/ml. The chromatograms obtained were recorded as represented in Figure 7 and 8.

4.8. Robustness

By analyzing robustness, resulted values were found to be within limit that is less than 2%, thus the developed method was proved to be robust. The results are summarized in Table 6.

4.9. Assay

The results obtained show that the percentage recoveries were high and SD values are very low, which confirms that the method is suitable for routine analysis of lemborexant in its pharmaceutical preparation. The results are summarized in Table 7.

Table 3 Method Precision (Assay Repeatability) data of Lemborexant

Sample No.	% Assay
1	95.46
2	94.84
3	97.49
4	97.63
5	97.55
AVERAGE	96.594
SD	1.337209782
%RSD	1.384361121
Limit	NMT 2%

Table 4 Intermediate precision data of Lemborexant

Sample No.	Day-1 HPLC-1	Day-2	HPLC-2
1	96.41	96.13	100.21

Table 5 Result of accuracy studies of lemborexant

Level	Found	Recovery	% Recovery	Average	Std dev	% RSD	Mean Recovery
	mg						(%)
	10.16	101.6	101.6				100.71
100%	10.13	101.3	101.3	101.3	0.139	0.137	
	10.14	101.4	101.4				
	11.09	110.9	100.8				
110%	11.1	111	100.9	100.9	0.059	0.058	
	11.09	110.9	100.8				
	11.98	119.8	99.8				
120%	11.99	119.9	99.9	99.9	0.047	0.047	
	11.99	119.9	99.9				
	13.05	130.5	100.4				
130%	13.04	130.4	100.3	100.3	0.521	0.52	
	13.16	131.6	101.3				
					Mean	0.1905	

Table 6 Robustness data of Lemborexant

Parameter	Change in parameter (±)	% Assay Estimation	AVERAGE	SD	% RSD	LIMIT
Flow rate (±0.2 ml/min)	0.8	94.96	94.57333333	0.335012438	0.354235624	NMT 2%
	1	94.39				
	1.2	94.37				
Column temperature (±5°C)	37	94.54	95.30666667	0.713045113	0.748158695	
	40	95.43				
	43	95.95				
Wavelength (±2 nm)	279	94.19	93.88333333	0.305013661	0.324885845	
	281	93.58				
	283	93.88				
Mobile Phase (±2)	58:42:00	96.13	96.26	0.147309199	0.153032619	
	60:40:00	96.23				
	62:38:00	96.42				

Table 7 Assay results of lemborexant tablets

Sample No.	Weight of standard (mg)	Sample weight (equivalent to 10 mg of Lemborexant)	Mean Area of the standard at 281 nm	Area of a sample at 281 nm	% Assay
1	10.04	129.7	1534416.6	1465832	95.46
2		130.33		1463333.7	94.84
3		129.78		1497905.7	97.49
4		129.73		1499586	97.63
5		129.21		1492349	97.55
				Mean	96.59
				± SD	1.34
				% RSD	1.38

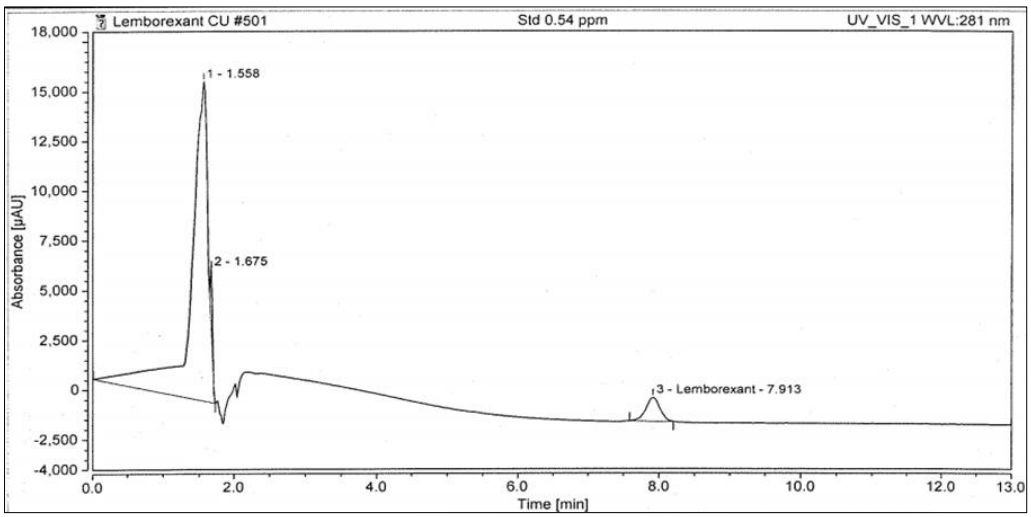


Figure 7 Chromatogram of LOD

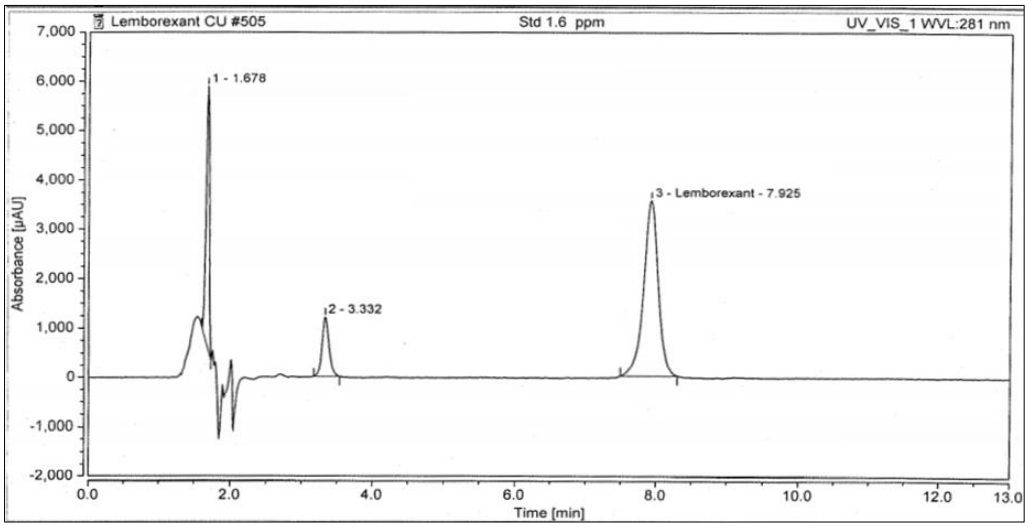


Figure 8 Chromatogram of LOQ

5. Conclusion

The RP-HPLC method development was found to be simple, precise, rapid, accurate for the quantification of Lemborexant in its tablet dosage form. The method was reliable in terms of system suitability, linearity, precision, accuracy and recovery, robustness, and assay. All the verification parameters were within the range according to ICH Q2A (R1) guidelines. Hence, authors conclude that the proposed RP-HPLC method can be used for routine analysis of lemborexant in the pharmaceutical industry.

Compliance with ethical standards

Acknowledgments

The author is thankful to The Director of Central Drug Testing Laboratory, Mumbai and Gahlot Institute of Pharmacy, Koparkhairane for the guidance and support. Special thanks to Dr. Vijay Kumar, Dr. Smita Nayak and Dr. V.H. Bhaskar. The authors are highly indebted to Smt. S.U. Warde, Mrs. Harshada Talapadatur and others for their valuable guidance and support to carry this research work.

Disclosure of conflict of interest

No conflict of interest to disclosed.

Abbreviations

RP-HPLC: Reversed Phase High Performance Liquid Chromatography;
LOD: Limit of detection;
LOQ: Limit of quantification;
ICH: International Council for Harmonization;
USA: United States of America;
UV-VIS: Ultraviolet- visible spectrophotometry;
SST: System suitability; SD: Standard deviation;
%RSD: Percentage relative standard deviation;
NMT: Not more than; BSD: Base deactivated silica;
API: Active pharmaceutical ingredient; SE: Standard error;
US FDA: United States Food and drug administration;
OX1R: Orexin 1 receptor antagonist; OX2R:
Orexin 2 receptor antagonist.

Summary

In the current study, a simple and novel isocratic high-performance liquid chromatography (HPLC) method was developed for quantitative determination of lemborexant in bulk as well as in its tablet dosage form. The developed HPLC method was validated as per ICH (Q2R1) guideline. The method validation involves various validation parameters such as system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection and limit of quantification. The results of validation parameters indicate that developed HPLC method was specific, accurate, precise, rapid, reliable and reproducible. Therefore, it can be applied.

References

[1] Scott LJ. Lemborexant: First Approval. Drugs. 2020; 80(4): 425-32.

[2] Wang C, Wang Q, Ji B, Pan Y, Xu C, Cheng B, Bai B, Chen J. The orexin/receptor system: molecular mechanism and therapeutic potential for neurological diseases. Front Mol Neuronsci. 2018; 11: 220.

[3] Beuckmann CT, Suzuki M, Ueno T, Nagaoka K, Arai T, Higashiyama H. *In vitro* and in silico characterization of lemborexant (E2006), a novel dual orexin receptor antagonist. J Pharmacol Exp Ther. 2017; 362(2): 287-95.

[4] Murphy P, Moline M, Mayleben D, Rosenberg R, Zammit G, Pinner K, Dhadda S, Hong Q, Giorgi L, Satlin A. Lemborexant, a dual orexin receptor antagonist (DORA) for the treatment of insomnia disorder: results from a Bayesian, adaptive, randomized, double-blind, placebo-controlled study. J Clin Sleep Med. 2017; 13(11): 1289-99.

[5] Grandner MA, Perlis ML. Pharmacotherapy for insomnia disorder in older adults. JAMA Netw Open. 2019; 2(12): e1918214.

[6] Rosenberg RP. Sleep maintenance insomnia: strengths and weaknesses of current pharmacologic therapies. Ann Clin Psychiatry. 2006; 18(1): 49-56.

[7] Ohayon MM, Reynolds CF 3rd. Epidemiological and clinical relevance of insomnia diagnosis algorithms according to the DSM-IV and the International Classification of Sleep Disorders (ICSD). Sleep Med. 2009; 10(9): 952-60.

[8] Available from: https://en.wikipedia.org/wiki/Lemborexant [cited 20/7/2021].

[9] Available from: https://www.rxlist.com/dayvigo-drug.htm [cited 20/7/2021].

[10] Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Lemborexant [cited 20/7/2021].

[11] Muggu, Murali Krishna S Naga Valli, Pushpa Anjali, P. Balamani Deep, D. Teja, J. Prem Kumar Naik. Method Development and Validation of Lemborexant in Bulk and its Pharmaceutical Dosage Form by (RP-HPLC). WJPR 2020, 1372-1380.

[12] Available from: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf.