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# High Performance Thin Layer Chromatography Method Development and Validation for Determination of Lemborexant in Pharmaceutical Dosage Form

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**ABSTRACT**
A simple and sensitive high performance thin layer chromatography (HPTLC) method was developed and validated as per the International Council for Harmonization (ICH) guidelines for the estimation of lemborexant in pharmaceutical dosage form. Chromatography was performed on 60 $F_{254}$ precoated TLC plates using toluene: methanol: ethyl acetate (6:2:2 v/v) as mobile phase, and densitometry detection was carried out at 281 nm for the quantitative estimation of the drug. The linear regression analysis data for calibration plots showed good linear relationship with $r^2$= 0.999 in the concentration range 200-1200 ng/band. The method was found to give compact band for the drug (Rf is 0.540±0.047). The range of recoveries was 98% to 102%. For Lemborexant, the limits of detection and quantitation were 6.35 and 19.25 ng/spot, respectively. In compliance with ICH requirements, the developed approach was validated. Statistical analysis of the data showed that the method is precise, accurate, reproducible and selective for the analysis of Lemborexant. The method was successfully used for the estimation of lemborexant as a bulk drug and in commercially available formulation.

**KEYWORDS**
*Lemborexant, analytical method, high performance thin layer chromatography (HPTLC), development, Validation*

## I. Introduction

Lemborexant is chemically (1R,2S)-2-[(2,4-Dimethylpyrimidine-5-yl) oxymethyl]-2-(3-fluorophenyl)-N-(5-fluorophenyl-N-(5-fluoropyridine-2-yl) cyclopropane-1-carboxamide (Fig. 1). Lemborexant is considered as a dual orexin receptor antagonist (DORA), exerting its sedative effects by reversible competitive binding to, and thus inhibiting, the wakefulness effects of orexin on OX1R and OX2R, with a stronger affinity to OX2R and used for the treatment of Insomnia [1]. Lemborexant is a dual orexin receptor antagonist utilized in the therapy of Insomnia. The principle benefit of Lemborexant is promoting sleep by blocking the orexin receptor. Extensive literature review for the quantitative analysis revealed that various analytical methods have been reported for the estimation of Lemborexant. A simple and novel isocratic high-performance liquid chromatography (HPLC) method for the estimation of Lemborexant was in bulk as well as in its tablet dosage form has been reported [2-6]. Lemborexant is not official in any pharmacopoeia. The HPTLC method provides accurate and precise results which are comparable to that of liquid chromatographic method. Reduced sample preparation methods, less analysis time, and small quantity of mobile phase required are some of its advantages over liquid chromatography. Densitometric scanning used in HPTLC for quantitative analysis offers advantages of accuracy, precision, and specificity over conventional methods used in TLC. Stability and degradation samples can also be analysed using a densitometer. HPTLC has become part of many pharmacopeial monographs for the estimation of the drug and impurities. So, the present study involves development and validation of Analytical methods for estimation of Lemborexant in pharmaceutical dosage form.

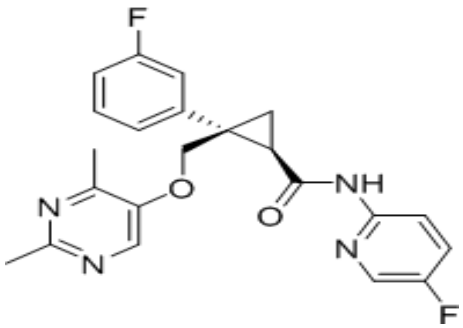


Fig 1 Chemical structure of Lemborexant

High-performance thin-layer chromatography (HPTLC) is an automated variation of thin-layer chromatography (TLC) with smaller sorbent particles and pores, shorter analysis times, and more efficient development chambers that use less mobile phase. Sample application in HPTLC is automated, and a UV/Visible/Fluorescence scanner—a sophisticated kind of densitometer—scans the whole chromatogram both qualitatively and quantitatively [7]. Its benefits, which include improved separation efficiency and detection limits, lower analysis costs, shorter analysis times, no need for solvent pre-treatment (such as filtration and degassing), lower mobile phase consumption per sample, and the elimination of interference from prior analyses because each analysis uses a fresh stationery phase and mobile phase, have made it increasingly important in the pharmaceutical industry. Additionally, the technique permits the determination of up to 18 sample sites to be applied in a single run. It is vitally crucial to establish a quick, easy, affordable, accurate, and exact analysis method like HPTLC in order to control the quality of Lamborexant. For the estimation of LAMB in their combined dose form, no HPTLC approach has been described.

## II. Method

**Experimental materials**

**Reagents and Chemicals**: Pure sample of LAMB was kindly supplied as gift sample by Zydus Pharma Ltd. HPLC grade methanol and analytical reagent grade Toluene, ethyl acetate and distilled water were used. Ethyl acetate, methanol and toluene was used in preparing the mobile phase. Tablet formulations containing Lamborexant 10 mg purchased from local pharmacy in Vadodara.

**Instrumentation and chromatographic condition:** Camag HPTLC apparatus consisting of Linomat V Sample applicator (Camag, Muttenz, Switzerland), 100 µL syringe (Hamilton-Bonaduz Schweiz, Camag, Switzerland), TLC Scanner III (Camag, Muttenz, Swtzerland), VisionCATS version 1.4.8 software (Camag, Muttenz, Switzerland) were used in the study Chromatography was performed on Merck silica gel 60 F254 precoated TLC plates (20 cm x 20 cm with 200 mm thickness; batch numbers: HX389048 and HX398477), Saturation pad (Camag, Muttenz, Switzerland) was used for saturating development chambers. Samples were applied as bands under a stream of nitrogen using the 100 microliter syringe (Hamilton-Bonaduz Schweiz, Camag, Swtzerland). Ascending development to a distance of 7 cm was performed in a 30 min presaturated 20 x 20 cm twin trough TLC developing chamber (Camag). Developed plates were dried using hair drier. Densitometry scanning and quantitative evaluation were performed using the TLC scanner and VisionCATS software, respectively.

**Preparation of standard solution:** A standard solution of LAMB was prepared by dissolving a quantity of 100 mg of Lamborexant in 100 ml of methanol in 100 ml volumetric flask make up with methanol. From this take 10 ml of solution and diluted upto mark with methanol (100 µg/ml).

**HPTLC Method and Chromatographic Conditions**

**Sample Application**

On precoated TLC plates, the standard and formulation samples of LAMB were identified as thin bands measuring 6 mm in length, 15 mm from the left and bottom margins, and 10.4 mm apart from one another. Samples were applied at a constant 150 nL/s under a continuous nitrogen gas drying stream.

**Mobile Phase and Migration**

The mobile phase used to develop the plates was Toluene: Methanol : Ethyl acetate (6: 2: 2 v/v/v). The development process was linear ascending in a 10 cm × 10 cm twin trough glass container that was mobile phase equilibrated. At 25 ± 2 °C, the ideal chamber saturation time for the mobile phase was 20 minutes. For every development, ten milliliters of the mobile phase (5 mL in the trough holding the plate and 5 mL in another trough) were used, and it took 10 min for the mobile phase to migrate 70 mm. Following development, the TLC plates underwent thorough drying.

**Densitometric Evaluation and Quantification Method:**

VisionCATS planar chromatography version 1.3.4 was used to control the Camag TLC scanner III in absorbance mode during densitometric scanning. The deuterium lamp was the radiation source that was used. The spots were examined at 281 nm in wavelength. The analysis employed slit size of 5 mm for length and 0.45 mm for width, with a scanning rate of 20 mm/s. These are chosen in accordance with the CAMAG TLC Scanner III manual's recommendations. It covers between 70 and 90 percent of the application band length, or 6 mm in this instance. A 20 nm bandwidth was used for the monochromator. The intensity of diffusely reflected light was used to calculate the concentrations of the substance chromatographed, and peak areas were compared to concentrations utilizing linear regression equation.

**Method validation**

The International Conference on Harmonization (ICH) criteria Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness were followed in the validation of the developed HPTLC method [8].

**Specificity**

By examining sample solutions containing LAMB from commercialized tablets in relation to interferences from formulation constituents, the specificity of the suggested approach was established. The confirmation of the LAMB spot in the sample was achieved by comparing the retardation factor ($Rf$) values of the spot with that the standard.

**Sensitivity**

The method's sensitivity was assessed in relation to the limits of quantification (LOQ) and detection (LOD). Six scans of the blank spot (methanol) were used to identify noise. To ascertain LOD and LOQ, a range of drug solution concentrations (200-1200 ng/spot) were put to a plate and examined. Three times the noise level was used to determine LOD, and ten times the noise level was used to calculate LOQ. By dilution of the known concentrations of LAMB until the average responses were roughly 3–10 times the standard deviation (SD) of the responses for six repeat measurements, LOD and LOQ were experimentally validated.

**Linearity**

In order to assess the method's linearity, calibration curves at six concentration levels were built. Throughout the concentration range of 200-1200 ng/band, calibration curves were drawn. Aliquots of LAMB standard working solution (2, 4, 6, 8, 10, 12 µL/band) were applied to the plate. Using the VisionCATS program, peak area versus concentrations ($n$ = 6) was plotted to create the calibration curves.

**Precision**

Both intra- and inter-day precisions were used to assess precision. Bands were applied to the HPTLC plate using a working standard solution containing 100 µg/mL LAMB for the precision investigation. By examining a solution of LAMB (200, 600,1200 ng/band) at three levels covering the low, middle, and high concentrations of the calibration curve three times on the same day (n = three), intra-day precision was ascertained. The analysis of a solution of LAMB (200, 600,1200 ng/band) at three levels covering low, medium, and high concentrations over a period of three days (n = 3) was used to evaluate the inter-day precision. The mean and relative standard deviation (% RSD) values were computed using the acquired peak regions. In order to assess the injection's repeatability, the peak area measurement's repeatability was assessed by evaluating the LAMB sample (600 ng/band) six times without moving the plate's location.

**Accuracy**

To confirm the accuracy of the proposed method, recovery experiments were carried out by the standard addition technique. It was carried out by adding known amounts of drug (reference standard) to samples of Lamborexant tablet corresponding to three concentration levels (80, 100, and 120% of the working concentration) along with the excipients and to the working standard.

**Robustness**

Modest adjustments were made to the mobile phase composition, solvent migration distance and chamber saturation duration, and the impact on the outcomes was assessed. The method's robustness was assessed at 600 ng/band of LAMB concentration level. The peak areas' mean and percentage RSD values were computed.

**Examination of the formulation**:

**Preparation of sample solution:** Lamborexant tablets was weighed and triturated. Powder equivalent to 10 mg Lamborexant was weighed accurately and transferred to 10 ml volumetric flask and volume was made up to mark with methanol. This solution was used as 1º stock solution (1000 µg/ml of Lamborexant). From this take 10 ml aliquot and diluted upto 100 ml with methanol (100 µg/ml**)**.

## III. Results and Discussion

The mobile phase was chosen based on polarity in order to develop the HPTLC method for the determination of LAMB. It is desired to have a mobile phase that yields a compact and dense band with a suitable Rf value for LAMB. In the early stages of developing the approach, several mobile phases were tested. Initially, Chloroform, methanol, toluene, glacial acetic acid and ethyl acetate were attempted as the mobile phase. The following combinations were tried as mobile phases: Ethyl acetate: methanol: glacial acetic acid (6: 4: 0.5, v/v/v), Ethyl acetate: methanol (6.5:3.5, v/v), Ethyl acetate :methanol: ammonia (4.5:5.5:0.5 v/v/v), ), methanol-toluene-glacial acetic acid (5:4:1, v/v/v), and ethyl acetate-toluene-acetic acid (6:3:1, v/v/v). Of them, toluene: methanol: ethyl acetate (6:2:2 v/v) solvent combination produced excellent LAMB separation from its matrix, with an Rf value of 0.54. Additionally, it was noted that solvent migration distance and chamber saturation time are important factors in chromatographic separation, with solvent migration lengths larger than 70 mm and chamber saturation times of fewer than 15 minutes resulted in the analyte spot diffusing. Consequently, the solvent system of toluene: methanol: ethyl acetate (6:2:2 v/v) proportion was employed as the mobile phase, with a solvent migration distance of 70 mm and a chamber saturation period of 20 min at 25°C. The lowest quantity of drug that could be measured under the experimental conditions used was 19.25 ng/band, while the lowest amount that could be detected was 6.35 ng/band. Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of sample matrix. LAMB was separated from excipients with an Rf value of 0.54± 0.04. There was no interfering peak at Rf value of LAMB so, no interference from excipients, present in commercial formulation, thereby confirming specificity of method. Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. Method was found to be linear in a concentration range of 200-1200 ng/ban (n=6), with respect to peak area. The regression data as shown in Table 1 reveal a good linear relationship over the concentration range studied demonstrating its suitability for analysis. Accuracy of an analytical method is the closeness of test results to true value. It was determined by the application of analytical procedure to recovery studies, where known amount of standard is spiked in preanalysed samples solutions. Results of accuracy studies from excipient matrix were shown in Table 2; Recovery values demonstrated the accuracy of the method in the desired range. The precision of an analytical method express the degree of scatter between a series of measurement obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of analytical method within a laboratory over a short period of time with the same instrument whereas Interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days. The results obtained are shown in table 3. In all instances, %RSD values were less than 2 confirming the precision method. Intraday and interday variations were performed by analysis of three different concentrations (200, 600 and 1200 ng/spot) of the drug three times on the same day and on different days, respectively. In order to control scanner parameter, that is repeatability of measurement of peak area, one spot as analysed without changing position of plate (n=6). By spotting and analysing the same amount several times (n=6), precision of automatic spotting device was evaluated. % RSD was consistently less than 2 (Table 4), which was well below the instrumental specifications, ensuring repeatability of developed method as well as proper functioning of the HPTLC system. The low values of %RSD (Table 5) obtained after introducing small deliberate changes in developed HPTLC method confirmed the robustness of the method. A single spot at Rf 0.54 was observed in the chromatogram of LAMB. No interference from the excipients present in the marketed tablet formulation observed. Analysis of LAMB tablets showed a drug content of 10.09± 0.12 (Table 6). The low %RSD value indicated the suitability of this method for routine analysis of LAMB in various formulations.

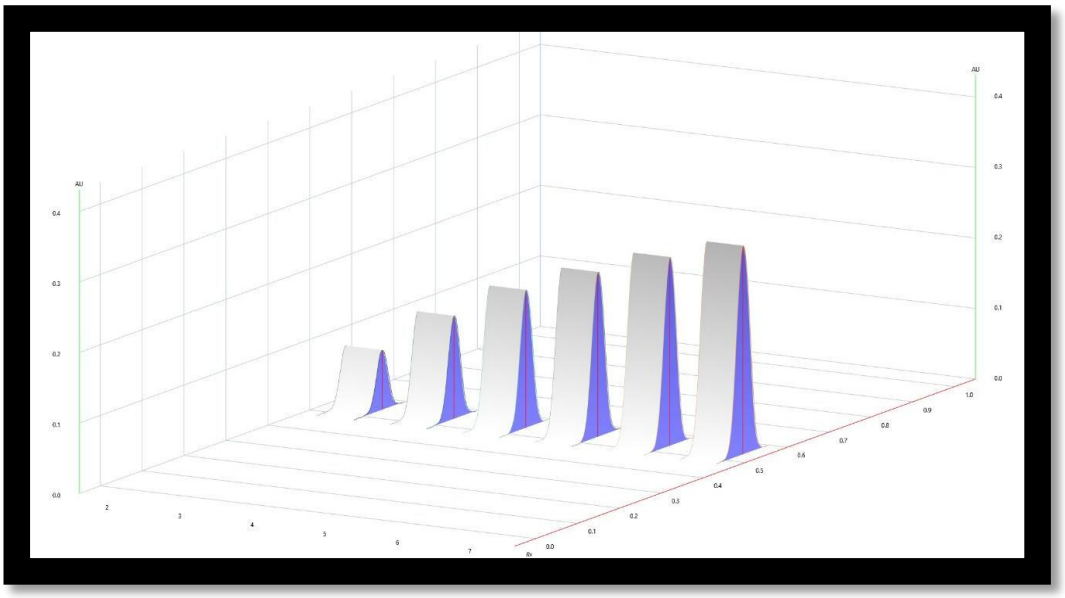

Figure 2: 3-D Chromatogram of LAMB (200-1200 ng/band) in optimized mobile phase

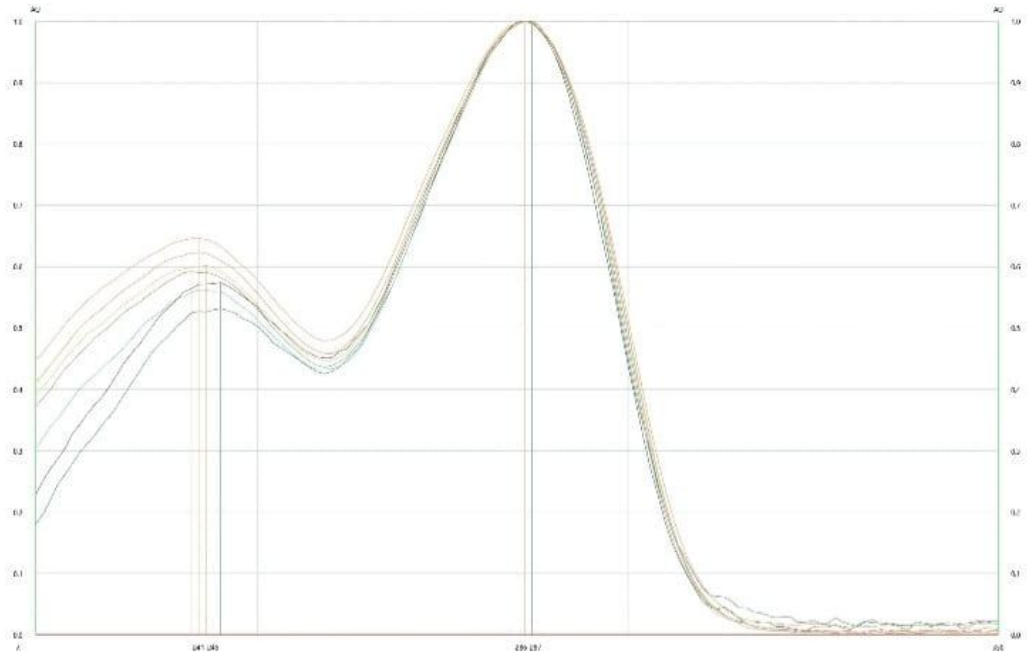

Figure 3: Overlain UV spectra of standard and sample LAMB

**Table I: Linear regression data for the calibration curves (n = 6).**

| Range (ng/band) | r2 | Slope ± SD | Intercept ± SD |
|---|---|---|---|
| 200-1200 | 0.999 | 0.7443± 0.003 | 239.57±1.43 |

**Table II: Recovery studies (n = 9)**

| Level % | Amount of drug analysed (ng/band) | Std concentration spiked (ng/band) | Total concentration found (ng/band) | Spiked concentration recovered (ng/band) | % recovery | % Mean recovery ± SD | %RSD |
|---|---|---|---|---|---|---|---|
| | 400 | 320 | 720 | 316.85 | 99.02 | | |
| 80 | 400 | 320 | 720 | 319.84 | 99.95 | 99.95±0.93 | 0.93 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 400 | 320 | 720 | 322.82 | 100.88 | | |
| | 400 | 400 | 800 | 403.48 | 101.30 | | |
| 100 | 400 | 400 | 800 | 401.46 | 100.37 | 100.34±0.97 | 0.97 |
| | 400 | 400 | 800 | 397.43 | 99.36 | | |
| | 400 | 480 | 880 | 484.13 | 100.86 | | |
| 120 | 400 | 480 | 880 | 475.34 | 99.03 | 99.59±1.10 | 1.11 |
| | 400 | 480 | 880 | 474.60 | 98.88 | | |

**Table III: Intra- and inter precision studies (n = 3)**

| Amount of drug spotted (ng/band) | Amount of drug detected (ng/band) (mean ± SD) | % RSD |
|---|---|---|
| **Intraday (n=3)** | | |
| 200 | 99.85 ±0.36 | 0.36 |
| 600 | 100.05± 0.09 | 0.09 |
| 1200 | 99.97 ±0.40 | 0.40 |
| **Interday (n=3)** | | |
| 200 | 99.94±0.65 | 0.65 |
| 600 | 100.03±0.35 | 0.35 |
| 1200 | 99.99±0.85 | 0.85 |

**Table IV: Repeatability Studies (n=6)**

| Amount of drug spotted (n=6) (ng/band) | Mean Peak area ± SD | %RSD |
|---|---|---|
| 600 | 689.5±2.42 | 0.35 |

**Table V: Robustness study**

| Parameters | Amount of LAMB spotted (ng/band) (n=3) | Amount of LAMB detected (ng/band) (n=3) Mean ± SD | %RSD |
|---|---|---|---|
| Mobile Phase composition Toluene:ethyl acetate:methanol: (5.5: 2.5 :2) | 600 | 100.08±0.45 | 0.45 |
| Mobile Phase composition Toluene:ethyl acetate:methanol: (6.5: 1.5 :2) | 600 | 99.97±0.58 | 0.58 |
| Migration distance (68 mm) | 600 | 99.94±0.71 | 0.71 |
| Migration distance (72 mm) | 600 | 99.95±0.93 | 0.93 |
| Chamber saturation time (15 min) | 600 | 100.04±0.77 | 0.77 |
| Chamber saturation time (25 min) | 600 | 100.98±0.39 | 0.39 |

**Table VI: Contents of LAMB in formulation (n=6)**

| Formulation | Label Claim | Amount Found (mean ± SD) | %RSD |
|---|---|---|---|
| F1 | 10 mg/tab | 10.01±0.31 | 1.29 |

## IV. Conclusion

A new HPTLC method has been developed for the identification and quantification of LAMB. Accurate, precise, economical and robust are the main features of this developed method. Method was successfully validated as per ICH guideline and statistical analysis proves that method is sensitive, specific and repeatable. It can be

conveniently used for routine quality control analysis of LAMB as bulk drug in marketed tablets without any interference from excipients.

## References


[1]. Lichstein, K. L., Taylor, D. J., McCrae, C. S., and Ruiter, M. E. Insomnia: Epidemiology and Risk Factors. In *Principles and Practice of Sleep Medicine, vol. 5, pp. 827-837, 2010*.

[2]. S.N Kamble, V. Munipalli, H. Talapadatur, R.M. Singh, S. Warde, S. Nayak and B. Vaidhun, "Development and validation of novel HPLC method for analytical evaluation of Lemborexant in tablet dosage form" GSC Advanced Research and Reviews, vol. 11(01), pp. 132–143, 2022.

[3]. R.M. Naik, S. Ahmed, S. Kumar, K. Radhika, K. Kumar," Development and Validation of UV-Spectroscopic methods for simultaneous Estimation of Hydrocortisone and Iodoquinol in Tablet Dosage Forms," Research J. Pharm. and Tech, vol. 13(8), pp 3777-3780, 2020.

[4]. Gunda Srija, "Practical Approach To High Performance Liquid Chromatography Method Development And Validation For Lamborexant In Tablet Dosage Form Or Bulk Or Any Other Pharmaceutical Dosage Form: Brief Review," International journal of research and analytical reviews, vol.9, June. 2022.

[5]. M. Muralikrishna, S. Nagavalli, P. Anjali, P. Balamani , D. Teja and J. Naik, "Method development and validation of lemborexant in bulk and its pharmaceutical dosage form by reverse phase–high performance liquid chromatography" World journal of pharmaceutical research, vol.9, pp.1372-1380, Nov. 2020.

[6]. B. Lakshmi Kalyani, V. Nikhila, J. Raga Swetha and B. Sushma Desai, "A comprehensive pharmaceutical analysis review on lemborexant drug," European Journal of Biomedical and Pharmaceutical sciences, vol.10, pp. 70-71, 2023.

[7]. Bose. "HPLC calibration process parameters in terms of system suitability test". Austin Chromatography, vol. 12, 2014.

[8]. ICH - International Council of Harmonization. "Stability testing of New Drug Substances and Products - Q1A (R2)" (2003).