UDC 539.3

OPTIMIZATION OF ROBUST HPLC APPROACH FOR ASSESSING PROCESS RELATED IMPURITIES OF LEMBOREXANT AND ELUCIDATION OF STRESS-INDUCED DEGRADATION PRODUCTS THROUGH LCMS/MS

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This study primarily aimed to establish an uncomplicated yet highly responsive HPLC technique to effectively separate and quantify process-related impurities of Lemborexant. Additionally, it aimed to explore the forced degradation behavior of lemborexant through systematic assessments by utilizing LCMS. The chromatographic separation of drug substance, process related impurities and its degradation products (DPs) was achieved on Kinetex C18 (150×4.6 mm, 5 µm) column at that was maintained at 35^0C temperature using 10 mM ammonium formate buffer pH 4.2, acetonitrile and methanol in 65:25:10 (v/v) isocratic elution at 0.7 mL/min. Detection wavelength was selected as 265 nm. In the proposed conditions, lemborexant is identified at 4.06 and 6.19, 9.33 min and 1.60 min respectively for impurity 1, 2 and 3 min with acceptable system suitability and specificity. The method produces LOD at 0.009 for impurities with calibration range of 40–280 µg/mL for lemborexant and 0.04 - 0.28 µg/mL for impurities. The remaining validation parameters were observed to fall within acceptable ranges for both lemborexant and its impurities. The compound underwent exposure to various stress conditions (acid, base, peroxide, thermal, and UV light) as outlined in accordance with ICH Q1A (R2) guidelines. The degradation products formed during the stress study were detected and characterized using LCMS/MS in ESI positive mode. This involved a thorough comparison of collision-induced dissociation mass spectrometry data between the degradation products and lemborexant. Consequently, potential structures for five degradation compounds were proposed. The outcomes of supplementary validation investigations were equally satisfactory, confirming their appropriateness for the regular quantification of lemborexant and its related impurities in both bulk drug and pharmaceutical formulations. Furthermore, these findings have the potential to shed light on the mechanism of stress-induced degradation in lemborexant.

Keywords: *Lemborexant, process related impurities, HPLC method development, forced degradation studies, degradation products identification.*

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Introduction

The existence of impurities within a pharmaceutical product can compromise its quality and lead to adverse effects. Factors such as the synthesis process, formulation choice, and storage conditions of a pharmaceutical compound can contribute to impurity formation [1]. To effectively identify these impurities within a drug compound, conducting impurity profiling becomes crucial. High-performance liquid chromatography (HPLC) methods that are capable of indicating stability play a pivotal role in quantitatively analyzing organic impurities present in drug substances [2]. Assessing the drug's stability under various environmental conditions over time holds significant importance. To accomplish this, the pharmaceutical industry

has embraced the guidelines outlined by the International Conference on Harmonization (ICH) [3–5]. Lemborexant is a pharmaceutical compound classified as a dual orexin receptor antagonist [6]. It is primarily used as a medication for the treatment of insomnia, a sleep disorder characterized by difficulty falling asleep or staying asleep [7]. Lemborexant acts by targeting and blocking the receptors for orexin, a neurotransmitter that plays a key role in regulating wakefulness and sleep [8]. By inhibiting the activity of orexin receptors, lemborexant helps to promote sleep and improve sleep continuity. Lemborexant is designed to be taken orally in tablet form and is usually administered before bedtime. It has shown efficacy in clinical trials for both reducing the time it takes to fall asleep (sleep onset latency) and increasing total sleep time. The drug's mechanism of action is distinct from that of traditional hypnotic medications, such as benzodiazepines, which target the GABAergic system. As with any medication, lemborexant may have potential side effects such as fatigue, headache, abnormal dreams and somnolence [9]. From review of literature, it was identified that two HPLC analytical methods reported for quantification of lemborexant in formulations [10, 11]. One *bio*-analytical LCMS method reported for quantification of lemborexant in biological samples and reported the pharmacokinetic profile in single [12] or simultaneously with three metabolites of lemborexant [13]. One UPLC–MS/MS method reported for quantification of lemborexant in plasma samples [14]. The extensive review of literature indicates the absence of any HPLC or LCMS-based stability assessment method validated in accordance with the ICH Q2(R1) guideline [3–5] for the evaluation of lemborexant and its impurities. In light of this gap, the present study aims to explore the degradation characteristics of lemborexant, identify its degradation products, and assess the stability of the lemborexant molecule under diverse stress conditions. This method also focused to resolve the process related impurities of lemborexant. The complete synthetic pathway for lemborexant involves several reactions such as substitutions, hydrolysis, amination, condensation, isomerization, protecttion and deprotection reactions [15]. During this synthetic process various chemical transformations and reactions take place. These proceses can sometimes lead to the formation of unintended impurities. Based on the availability, the impurity 1, 2, and 3 were selected in the study.

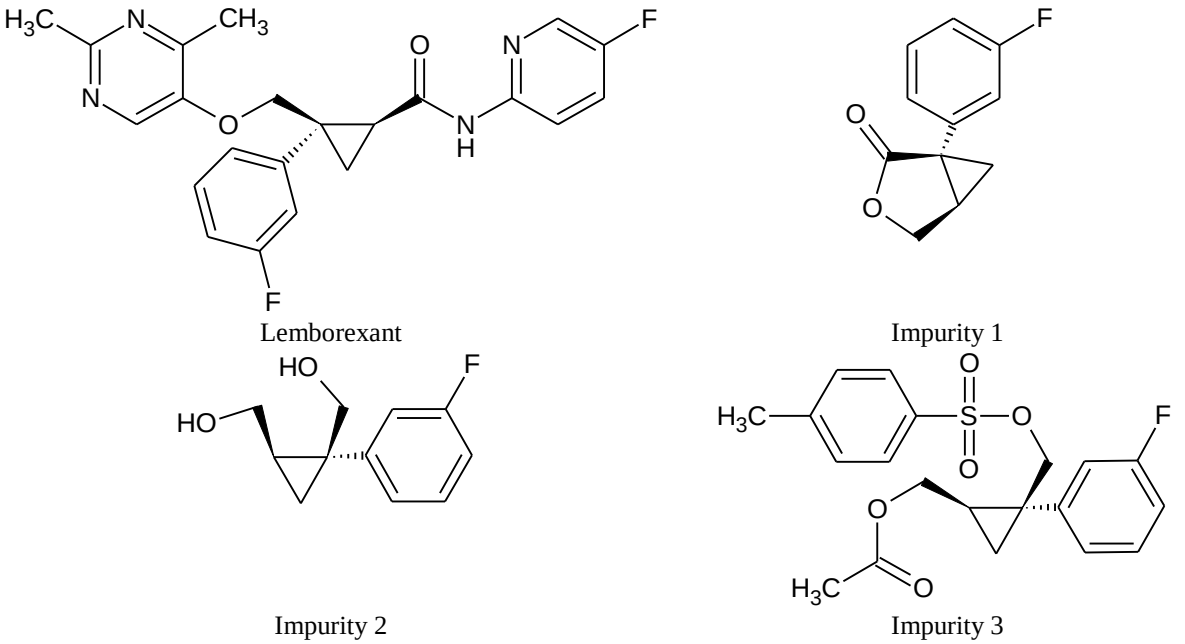


Fig. 1. Molecular structure of lemborexant and impurities.

Experimental procedure

Reagents and chemicals

The lemborexant active pharmaceutical ingredient, with a potency of 98.25%, was acquired from Lundbeck India Private Limited in Bengaluru, Karnataka, India. The process related impurities 1, 2, and 3 of lemborexant were also obtained from the same source. The Dayvigo® brand tablet formulation, containing 10 mg of lemborexant, was purchased from a local pharmacy. Milli-Q® water, 0.2 µ nylon membrane filters, as well as HPLC grade acetonitrile and methanol solvents, were sourced from Merck chemicals in Mumbai. LR grade reagents including sodium acetate, formic acid, hydrogen peroxide, sodium hydroxide (NaOH), and hydrochloric acid (HCl) used in this study were procured from Fisher scientific, Mumbai.

Instrumentation

The process of method development and validation was executed using an HPLC system (1100 series), consisting of a G1311 A model quaternary pump, G1329A model temperature-variable auto-sampler featuring an injection capacity of 0.1–1500 µL, and a G1314 A model ultraviolet (UV) detector. Chromatographic integrations were conducted using Agilent ChemStation software. Stress degradation compound assessment via LCMS was performed on a triple quadrupole LCMS instrument (Waters, Japan) operated with MassLynx software.

Preparation of solutions

Lemborexant and impurity solutions

Exactly 25 mg of pure lemborexant drug and its impurities were weighed and placed into a 25 mL flask already containing 15 mL of the diluent (pure methanol). Subsequently, an ultrasonic bath sonicator was employed to dissolve the measured analytes within the solvent, followed by filtering the solution through a 0.2 µm filter. The solution volume within the flask was then adjusted using the same solvent to attain separate solutions of lemborexant and its impurities, each with a strength of 1000 µg/mL. Appropriate dilutions were prepared from this stock solution where ever required.

Formulation solution

The Dayvigo® pharmaceutical formulation, containing 10 mg of lemborexant, was employed to create the formulation solution. One tablet of the formulation contains 10 mg of lemborexant. To prepare the solution, one tablet was accurately weighed and placed into a 10 mL volumetric flask, which was initially filled halfway with methanol. Sonication was applied to ensure complete dissolution of the analytes from the formulation into the solvent. The flask's volume was then adjusted to the mark using the same diluent, followed by filtration through 0.2 µm membrane filters to obtain a 1000 µg/mL sample solution. From this solution, the desired formulation concentration was subsequently prepared as needed.

Method development

The method development process initiated with the determination of an appropriate wavelength for the UV detector to detect lemborexant and its impurities. A UV-visible spectrophotometer was utilized to identify the wavelengths for both lemborexant and its impurities. To achieve this, a standard solution containing lemborexant and its impurities at a concentration of 10 µg/mL was individually scanned across the range of 400 to 200 nm. By overlaying the UV absorption spectra of lemborexant and its impurities, a wavelength with isoabsorption was confirmed, deemed suitable for precise detection of both lemborexant and impurities. Multiple stationary phase configurations from different manufacturers were examined to optimize the resolution of lemborexant and its impurities. Through systematic experimentation encompassing various solvent combinations, pH ranges, and flow rates, the composition of the mobile phase was refined. The conditions that effectively separated lemborexant and its impurities were selected and carried forward for subsequent validation procedures.

Method Validation

Method validation studies were conducted to assess the suitability of the proposed method, following ICH guidelines [3–5] and considering methods previously reported in lit-

erature [16–24]. The detection limit (LOD) and quantification limit (LOQ) were determined using a signal-to-noise ratio approach with specifications of 3:1 and 10:1 (s:n), respectively. LOD and LOQ provide insights into method sensitivity and lower results indicating higher sensitivity that was confirmed through sequential analysis of the lowest impurity concentrations.

For preparing calibration curve standard dilutions of lemborexant and its impurities, LOQ of impurities was taken into account. The lowest impurity concentration established the minimum calibration curve concentration, while lemborexant concentration considered its 0.1% impurity content. The solutions were evaluated in the proposed method, and a calibration curve was constructed plotting area (y-axis) against concentration (x-axis) through least squares regression.

Precision was evaluated to determine the repetition and reproducibility of results in the proposed method. Lemborexant solution containing 0.1% impurities was assessed on the same day (n=6), two alternate days (n=3), and by two different analysts (n=3) to ascertain intraday, interday precision, and ruggedness, respectively. Robustness was tested by introducing slight deviations intentionally, evaluating the solution's response to varied conditions with permissible changes of <2%.

Method accuracy was assessed by adding pre-analysed samples at different levels (LOQ, 50%, 100%, and 150%) within the linearity range. Recovery percentages and % RSD for each level were tabulated, meticulously analysed for accurate method assessment.

Stress degradation studies were conducted to evaluate the specificity and stability-indicating capacity of the optimized method. Placebo and lemborexant tablet powders were subjected to acid, base, and peroxide degradation (5 mL each of 1N HCl, 1N NaOH, and 3% hydrogen peroxide). Similarly, UV and thermal degradation studies were performed at 103.959 Wh/m² energy and 80°C for 24 hours. Stress-exposed samples were neutralized and prepared for analysis, and chromatograms were carefully observed for degradation products. Chromatographic response was compared with the calibration curve for % stability evaluation. Degradation compounds generated during stress exposure were identified and characterized through LCMS/MS analysis. UV-detected eluents were directed to a mass detector for mass spectral characterization, ensuring 40% eluents entered the mass detector using a splitter.

The proposed analytical HPLC method was investigated to identify and quantify lemborexant impurities in injection formulations. Sample solutions from Dayvigo® tablets were prepared and evaluated directly, spiked with known impurity concentrations. Chromatograms and responses observed in these assessments were used to evaluate the method's applicability.

Results and Discussions

Due to the absence of any documented analytical approach in the literature for quantifying the process related impurities of lemborexant, the primary objective of this study was to develop a straightforward HPLC technique. The method optimization was conducted using various column configurations, including octadecylsilane (ODS), phenyl–hexyl, cyano, and amino columns, to achieve the optimal resolution of analytes. The mobile phase composition with a suitable pH range was optimized by testing different solvent compositions, including various buffer strengths. As hydrophilic ionizable functional groups were present in both lemborexant and its impurities, pH buffer-containing mobile phases with different pH ranges were explored for effective resolution.

Effective separation of lemborexant and its impurities was achieved using a Kinetex C18 (150×4.6 mm, 5 μm) column, maintained at a temperature of 35^0C. The optimized chromatographic conditions consisted of a mobile phase composed of 10 mM ammonium formate buffer (pH 4.2), acetonitrile, and methanol in a 65:25:10 (v/v) ratio. Isocratic elution was performed at a flow rate of 0.7 mL/min. Detection was carried out at a wavelength of 265 nm, chosen based on significantly high detector response compared to other wavelengths for all analytes.

Under the proposed conditions, lemborexant and its impurities exhibited symmetrical

peak shapes in the chromatogram. The differentiation between adjacent peaks exceeded a value of 2, indicating clear separation. Identification of components within the reference solution involved analysing standard solutions individually and correlating their retention times with established standards. The determined retention times were 4.06 minutes for lemborexant, 6.19 minutes for impurity 1, 9.33 minutes for impurity 2, and 1.60 minutes for impurity 3. Regarding column efficiency, the tailing factor for both lemborexant and its associated impurities fell below the acceptable threshold, while theoretical plates and resolution values surpassed the acceptable limit. Detailed outcomes are documented in Table 1. Chromatograms presented in Figure 2 illustrated the placebo, standard lemborexant solution spiked with impurities, and the impurity separation and detection pattern. These chromatograms confirmed the method's specificity in effectively isolating and identifying process-related impurities of lemborexant.

The system suitability data illustrates the system's suitability, with a tailing factor below 1.5, a resolution surpassing 2.5 between adjacent eluting analyte peaks, and theoretical plates for all analyte peaks exceeding 2500. This outcome underscores the method's robust selectivity. Sensitivity assessment was conducted using a signal-to-noise (s/n) approach, with results expressed as the detection limit (LOD) and quantification limit (LOQ). The calculated LOD was determined as 0.007 µg/mL, while the LOQ for both impurity A and impurity B was found to be 0.025 µg/mL. These findings highlight the method's heightened sensitivity, making it particularly suitable for impurity quantification.

To construct the calibration curve for impurities, the LOQ concentration of 0.025 µg/mL was selected as the starting point. A lemborexant standard solution containing 0.1% of each impurity was prepared, establishing a precise calibration curve across the concentration range of 40–280 µg/mL for lemborexant and 0.04–0.28 µg/mL for the investigated impurities. The calibration curve displayed a robust linear correlation, evidenced by notably high correlation coefficients for both impurities and standard lemborexant.

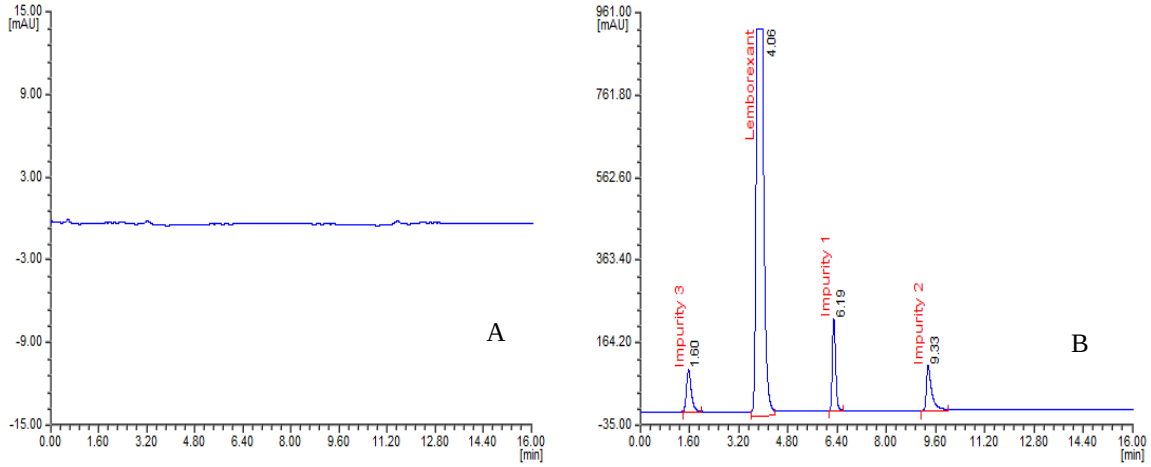
The obtained peak area values exhibited a relative standard deviation (%RSD) below the set threshold in various precision studies, including intraday and interday precision, precision at the LOQ, and ruggedness assessments, for both lemborexant and the two impurities. These results underscore the method's commendable precision.

To evaluate accuracy, recovery studies were performed by spiking concentrations of 50%, 100%, and 150% of the target levels– equating to 80 µg/mL for lemborexant and 0.08 µg/mL for impurities 1, 2, and 3. % Recovery was calculated for the standard and all impurities in each analysis, with %RSD values determined for each spiked level. Across lemborexant and the studied impurities, the achieved % recovery fell within the acceptable range of 98–102%, confirming the method's accuracy. Moreover, %RSD values at each spiked level remained below 2%, aligning with the acceptable limit and further affirming the method's accuracy. The summarized validation outcomes for the proposed method are presented in Table 1.

Minor deviations in the proposed method conditions did not result in any significant changes in the chromatographic response or system suitability. Resolutions between consecutive analytes consistently exceeded 2.0, and tailing factors for all analytes remained within acceptable limits. The variability in lemborexant and impurity estimation remained below the acceptable threshold of 2, confirming the method's robustness. Comprehensive results of the robustness study conducted within the developed method are provided in Table 2.

Forced degradation studies performed to evaluate the effectiveness of the method for resolution of degradation compounds and the study was conducted in acid, base, peroxide, thermal and UV light degradation conditions.

There is no considerable degradation was noticed in thermal degradation conditions with % assay of 97.97. Among the degradation conditions, high % degradation was noticed in acid degradation study with a % degradation of 8.41 %.



Placebo analysis chromatogram (A) and precision level solution of lemborexant spiked with impurities (0.1 %).

Fig. 2. Chromatograms observed in specificity and system suitability study.

Table 1. Summary results noticed in method validation

Parameter	Results			
	Lemborexant	Impurity 1	Impurity 2	Impurity 3
System suitability[$]				
t_R (min)	4.06	6.19	9.33	1.60
RRT	–	1.52	2.30	0.39
RRF	–	0.096	0.069	0.058
R_S	8.79	8.01	11.53	–
A_S	0.93	0.95	0.94	1.07
N	5813	6509	7818	9746
Linearity				
Range in µg/mL	40–280	0.04–0.28	0.04-0.28	0.04–0.28
Slope	6301.6	596659	532129	470933
Intercept	2990.9	793.49	68.014	- 147.16
r^2	0.9997	0.9994	0.9999	0.9996
Precision[$$]				
Intraday	0.43	0.30	0.45	0.39
Interday (day 1)	0.16	0.29	0.53	0.29
Interday (day 2)	0.72	0.39	0.31	0.34
LOQ level				
Accuracy at 50 % level[$]				
Amount added (µg/mL)	120	0.12	0.12	0.12
Recovered (µg/mL)	119.84	0.12	0.12	0.12
% Recovery	99.87	99.66	99.98	100.00
% RSD	0.44	0.37	0.14	0.15
Accuracy at 100 % level[$]				
Amount added (µg/mL)	160	0.16	0.16	0.16
Recovered (µg/mL)	158.67	0.16	0.16	0.16
% Recovery	99.17	99.74	99.87	99.84
% RSD	0.76	0.53	0.28	0.33
Accuracy at 150 % level[$]				
Amount added (µg/mL)	200	0.20	0.20	0.20
Recovered (µg/mL)	198.74	0.20	0.20	0.20
% Recovery	99.37	99.76	99.80	99.86
% RSD				
Sensitivity				
LOD (µg/mL)	–	0.007	0.007	0.007
LOQ (µg/mL)	–	0.025	0.025	0.025

The chromatogram observed in this study (figure 4A) show well resolved DPs at t_R of 6.16 min and 11.26 min and were named as DP 3 and DP 5 respectively. The chromatogram identified in base degradation study (figure 4B) clearly resolve two degradation products at t_R of 1.94 min and 4.96 min and were designated as DP 1 and DP 2 respectively with a % degradation of 6.99 %. The peak corresponds to impurity 2 at t_R of 9.33 min was also noticed in base degradation chromatogram. The % assay of lemborexant in peroxide degradation was calculated to be 6.25 % with mass balance of 99.13 %. The chromatogram clearly resolves two DPs at t_R of 4.96 min and 8.22 min and these impurities were marked as DP 2 and DP 4 respectively. The PDA detector's assessment of peak purity validated the purity and uniformity of the lemborexant peak across all stressed samples analyzed. The mass balance for the stressed samples ranged from 98.93% to 99.71%. These consistent peak purity test results reiterated the homogeneity and purity of the lemborexant peak within the examined stress samples. The lemborexant assay displayed minimal variation even in the presence of impurities, and the peak purity outcomes of the stress samples further affirm the specificity and capability of the developed method to indicate stability. Detailed information is provided in Table 3, while Figure 3 visually depicts representative chromatograms observed during the forced degradation study.

Table 2. Results observed in robustness study

S No	Changed condition	Parameter	Results observed			
			Lemborexant	Impurity 1	Impurity 2	Impurity 3
1	MP 1	% change	0.24	0.24	0.23	0.37
		t_R	4.01	6.18	9.32	1.65
		N	5807	6473	7848	9777
2	MP 2	% change	0.08	1.36	0.23	0.13
		t_R	4.09	6.15	9.36	1.62
		N	5828	6573	7843	9803
3	pH 1	% change	1.65	0.25	0.62	1.75
		t_R	4.03	6.12	9.37	1.67
		N	5754	6443	7573	9623
4	pH 2	% change	2.54	1.69	1.15	0.75
		t_R	4.01	6.16	9.32	1.63
		N	5780	6474	7946	9693
5	WL 1	% change	1.44	-0.63	0.48	0.03
		t_R	4.05	6.18	9.35	1.69
		N	5845	6531	7844	9803
6	WL 2	% change	0.83	0.25	0.75	0.78
		t_R	4.03	6.17	9.39	1.64
		N	5789	6474	7132	9684

MP (mobile phase) 1: 60:20:20 (v/v); MP 2: 65:30:5 (v/v); pH 1: 4.0; pH 2: 4.4; WL (wavelength) 1: 260 nm; WL 2: 270 nm; $^{\$}$average results (n=3)

Table 3. Forced degradation results of lemborexant

Condition	% degradation$^{\#}$ of lemborexant	% assay$^{\#}$ of lemborexant	% Mass balance$^{\$}$ (assay + total impurities	Remark
Acidic	8.41	91.59	99.37	DP 3 and 5 were noticed
Basic	6.99	93.01	99.13	DP 1 and 2 were noticed
Peroxide	6.25	93.75	99.12	DP 2 and 4 were noticed
Thermal	2.03	97.97	99.69	No degradation was identified
UV light	2.08	97.92	99.08	DP 1 was noticed

$^{\#}$average of three replicate experiments

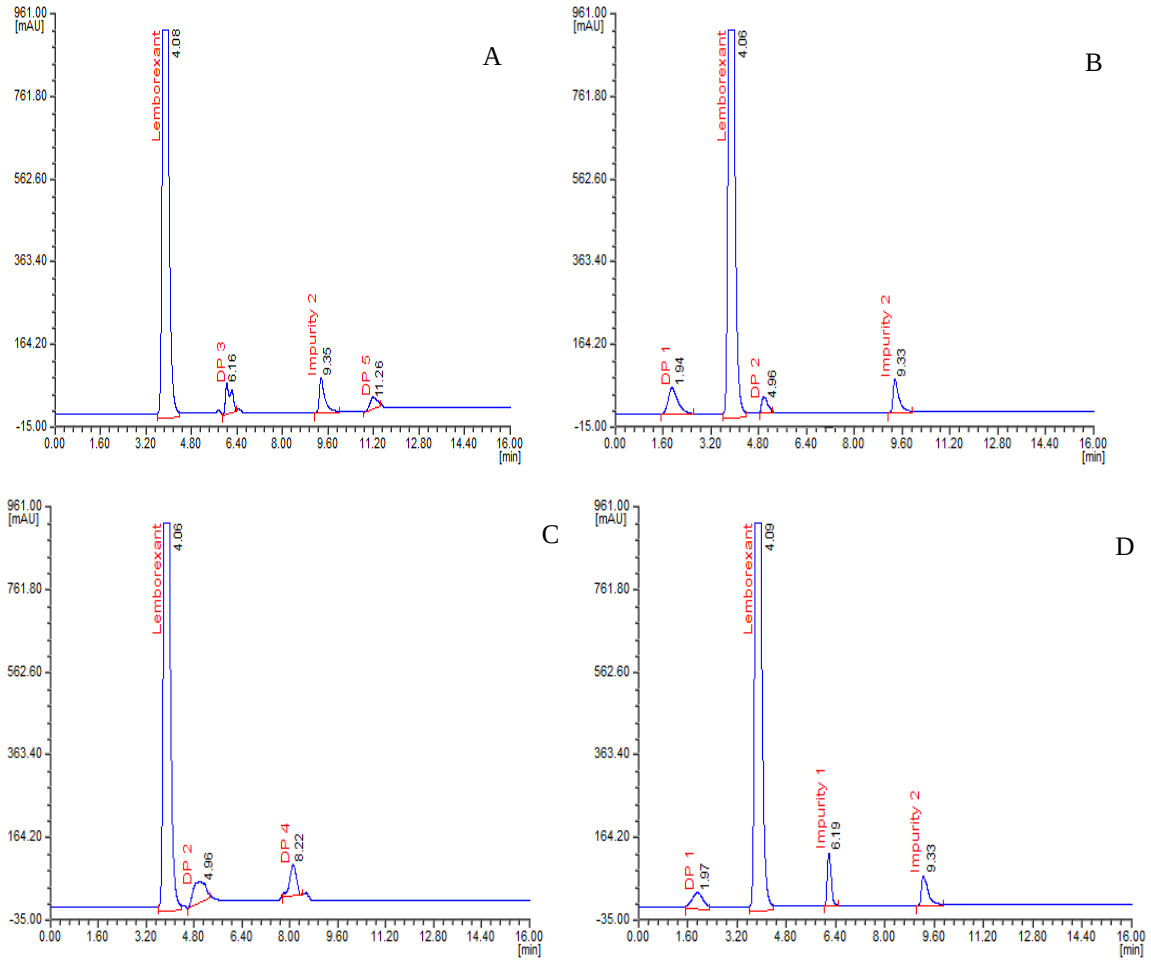


Fig. 3. Forced degradation chromatograms of lemborexant. A) Acidic stress study chromatogram of lemborexant visualizing DP 3 and 5; B) Basic stress study chromatogram of lemborexant visualizing DP 1 and 2; C) Peroxide degradation chromatogram of lemborexant visualizing DP 4 and 4 D) UV Light degradation chromatogram of lemborexant visualizing DP 1

Characterization of DPs by LCMS/MS:

The degradation products (DPs) resulting from the stress-induced impact on pure lemborexant drug were subjected to characterization using LCMS/MS analysis. The LC conditions optimized during the study were applied without modification, while the mass operating conditions were refined to ensure the production of maximum detection for each mass fragment while minimizing noise. The mass detector operated 3500 V of capillary voltage, 55 V of fragmentor voltage and 60 V of skimmer voltage, 5.5 L/H flow of drying (nitrogen) gas at 370^0C and 35 Psi of nebulizer gas. The same experiment condition was monitored throughout the analysis with average 20–30 scans were conducted. The preliminary test confirms that the positive ion mode was suitable for optimum and maximum detection of all DPs.

The ESI MS spectrum of DP 1 depicted in Figure 9A, identified at tR of 1.94 minutes, exhibits a prominent parent ion at m/z 317 (m+1), suggesting a plausible molecular formula of $C_{16}H_{17}FN_4O_2$. Additionally, the spectrum displays less abundant product ions at m/z 113 (m+1) with a molecular formula of $C_5H_5FN_2$. Based on the observed fragmentation pattern, the compound is recognized as *2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-N-(5-fluoropyridin-2-yl)cyclopropanecarboxamide*, characterized by a molecular formula of $C_{16}H_{17}FN_4O_2$ and a molecular mass of 316 g/mol. The proposed mass fragmentation pattern of DP 1 is illustrated in Figure 4.

The mass fragmentation spectra of DP 2 (Figure 9B) reveal a dominant parent ion at m/z 303 (m+1) when observed under positive ionization mode. Additionally, the spectrum dis-

plays fragment ions at m/z 149 (m+1) resulting from the loss of $C_7H_{10}N_2O_2$. Through accurate mass measurements, the elemental compositions of the molecular ion of DP-2 and all its fragmented ions have been verified. Based on the data obtained, DP 2 has been definitively identified as *[2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl) cyclopropyl]-methanol*, possessing a molecular formula of $C_{17}H_{19}FN_2O_2$. The proposed mass fragmentation pattern of DP 1 is depicted in Figure 5.

The ESI-MS spectrum of DP 3 (presented in Figure 10C), observed at a retention time of 6.16 minutes, displays a parent ion at m/z 316 (m+1) alongside a prominent fragment ion at m/z 149 (m+1). The parent ion's molecular formula is identified as $C_{17}H_{18}FN_3O_2$, and this corresponds to the fragment ion with a molecular formula of $C_{10}H_9F$ (resulting from the loss of $C_7H_9N_3O_2$). Further analysis reveals that the compound is characterized as *2-{[(2,6-dimethylidene-1,2,5,6-tetrahydropyrimidin-5-yl)oxy]methyl}-2-(3 fluorophenyl) cyclopropane carboximidic acid*. The structural details of this compound, as well as its associated fragmentation mechanism, are illustrated in Figure 6.

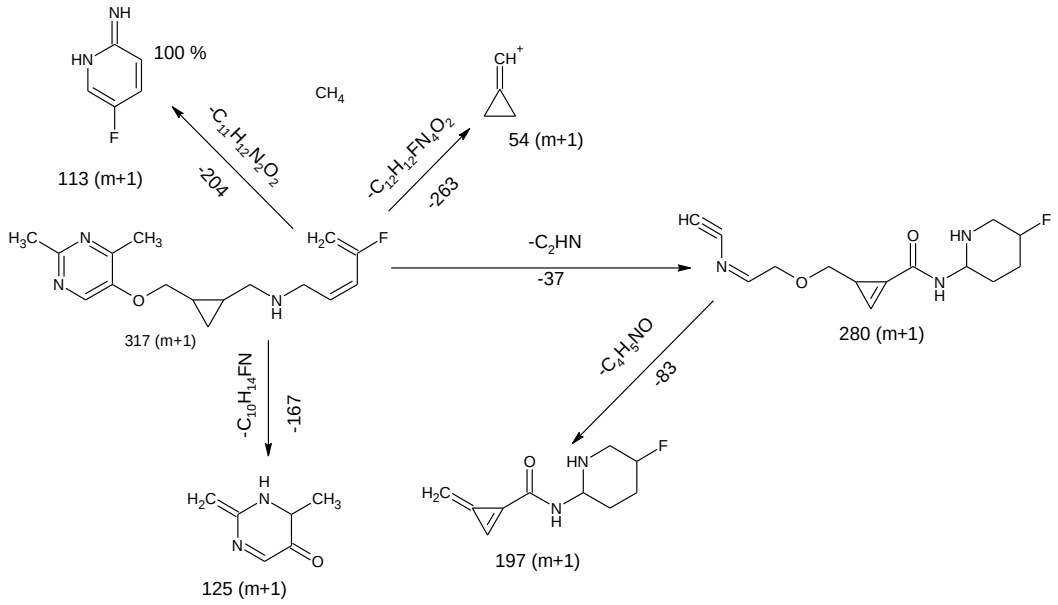


Fig. 4. Mass fragmentation pattern of DP 1.

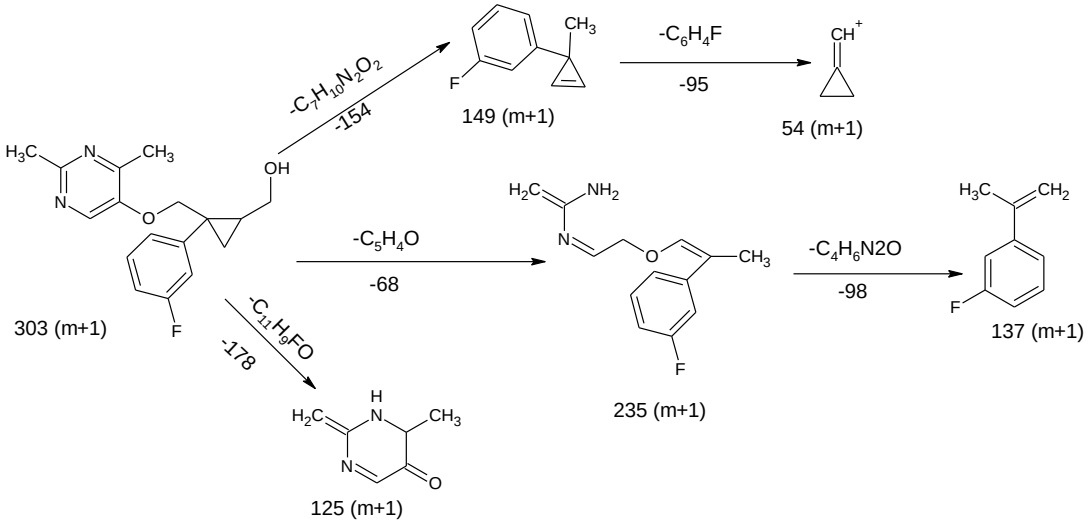


Fig. 5. Mass fragmentation pattern of DP 2.

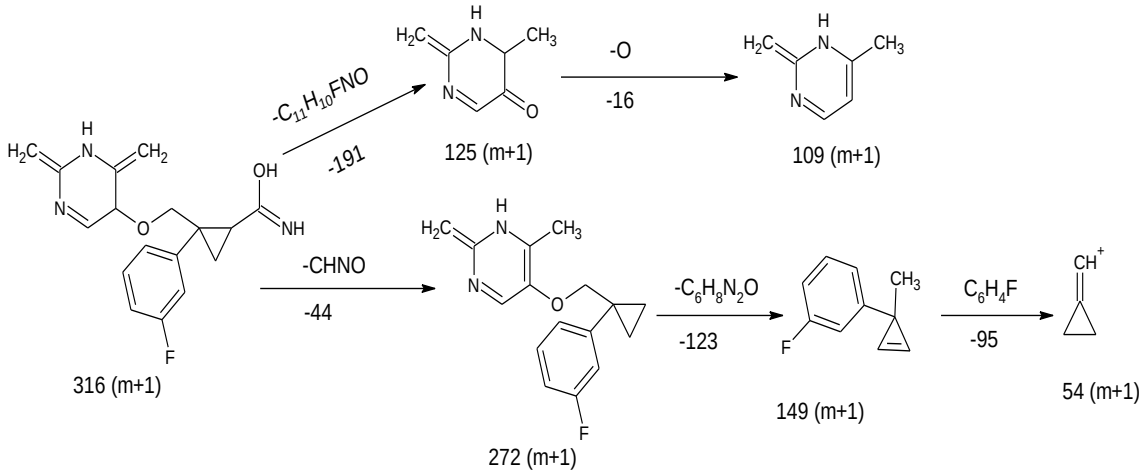


Fig. 6. Mass fragmentation pattern of DP 3.

The ESI-MS spectrum, observed at a retention time of 8.22 minutes (Figure 9D), exhibits a parent ion at m/z 355, corresponding to the $[M+H]^+$ of DP 4, which forms under peroxide stress conditions. Within the spectrum, there are abundant product ions at m/z 149 (m+1). The integrity of DP 4's molecular structure is validated through both peak purity testing and CID studies. The collection of these product ions, in conjunction with the parent ion, serves to affirm that DP 4 is indeed *2-({[(1E)-1-aminobut-1-en-2-yl]oxy}methyl)-2-(3-fluorophenyl)-N-(pyridin-2-yl)cyclopropanecarboxa–mide*, having a molecular formula of $C_{20}H_{22}FN_3O_2$. A representation of its structure, alongside the fragmentation mechanism is presented in Figure 7. The ESI MS spectrum of DP 5 (Figure 9E) displayed notable product ions at m/z 305 $[M+H]^+$. A significant product ion at m/z 125 possibly resulted from the loss of $C_{11}H_{13}FO$ from m/z 305. The elemental compositions of the molecular ion of DP 5 and all its fragmented ions were confirmed through precise mass measurements. The studies confirms that DP 5 was derived as a DP of DP 3 formed in study. Based on these analyses, DP 5 was identified as *4-[(2,6-dimethylidene-1,2,5,6-tetrahydropyri–midin-5-yl)oxy]-3-(3-fluorophe–nyl)-2-methyl–butan -1-ol* with a molecular mass of 304 g/mol and a chemical formula of $C_{17}H_{21}FN_2O_2$. Figure 8 present its molecular structure and fragmentation mechanism.

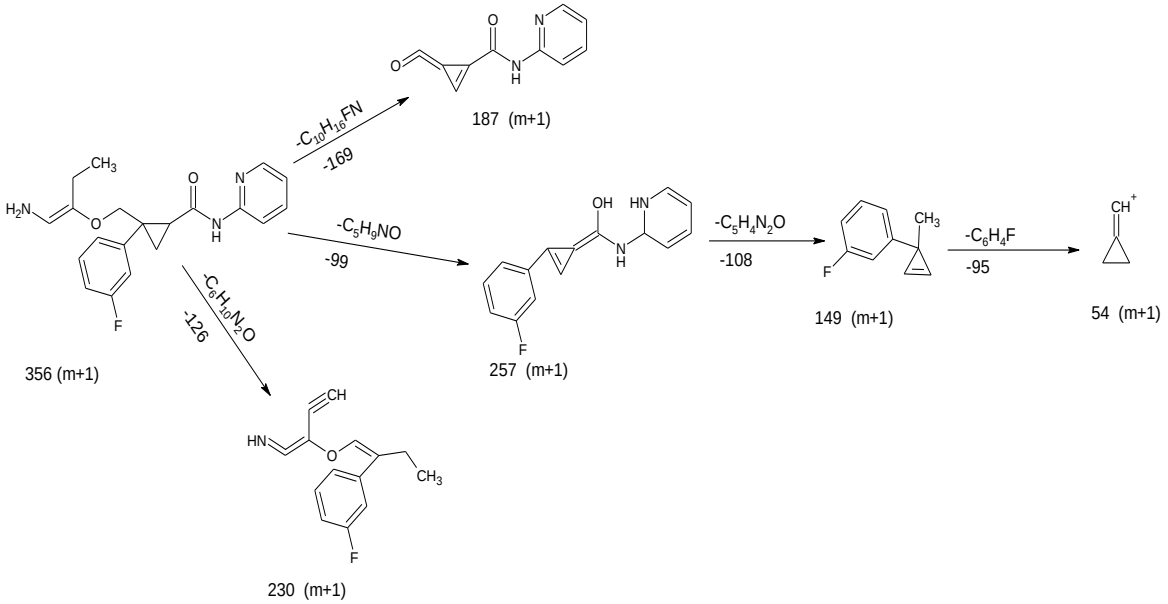


Fig. 7. Mass fragmentation pattern of DP 4.

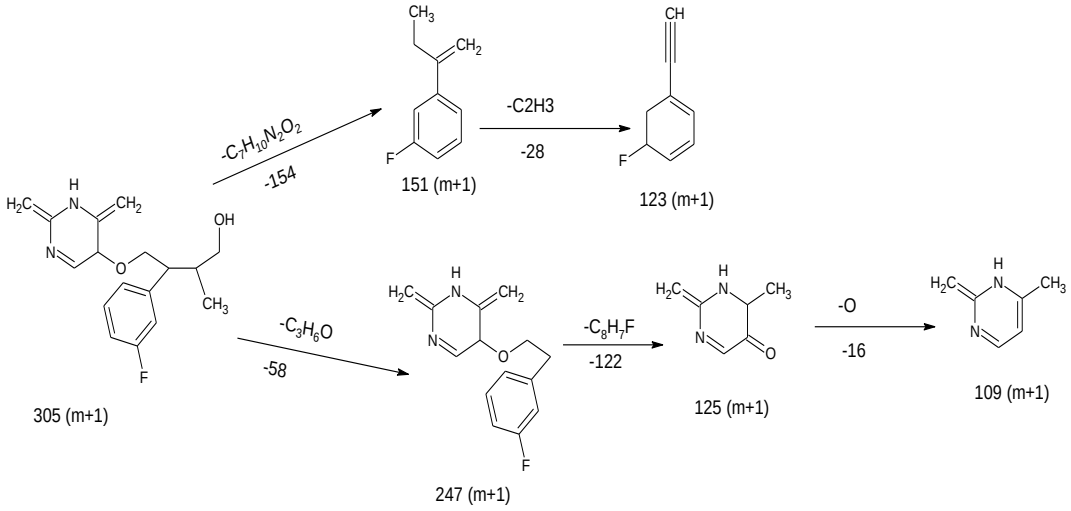


Fig. 8. Mass fragmentation pattern of DP 5.

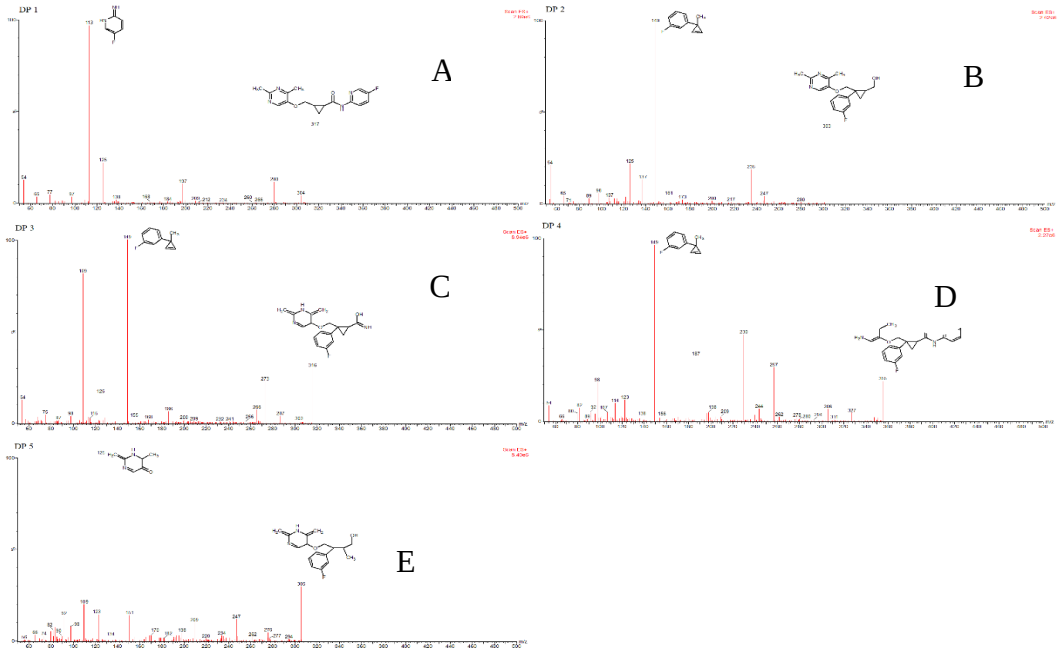


Fig. 9. Mass spectra of DPs observed in forced degradation study. Mass spectra identified at t_R of 1.94 min for DP 1 (A), 4.96 min for DP 2 (B), 6.16 min for DP 3 (C), 8.22 min for DP 4 (D), 11.26 min for DP 5 (E).

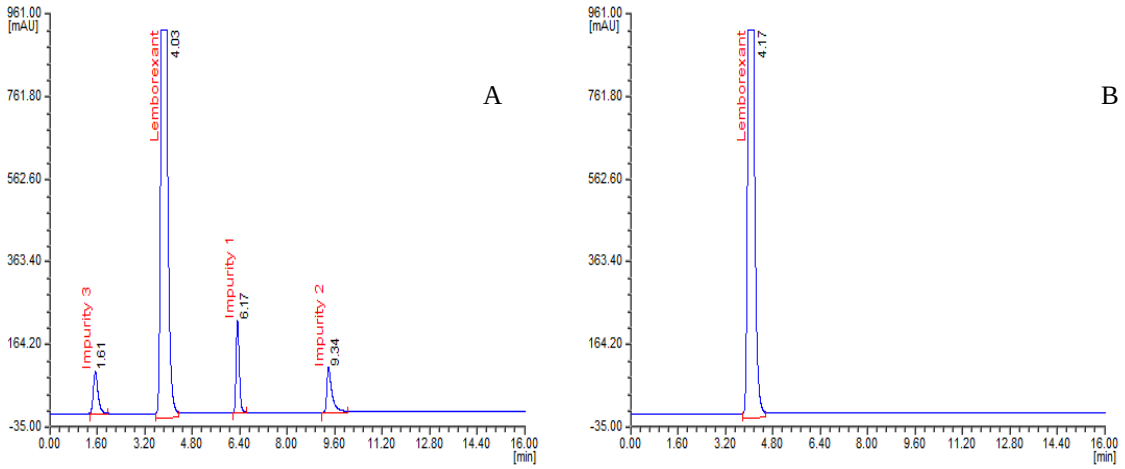


Fig. 10. Formulation analysis chromatogram of lemborexant. Chromatogram noticed for formulation solution spiked with impurities (A) and with no impurities spiked (B).

The established HPLC method was effectively employed for quantifying process related impurities of lemborexant within a pharmaceutical formulation. The formulation sample underwent direct analysis to evaluate the impurity content within it. Furthermore, a formulation sample spiked with impurities was examined to assess the method's ability to separate and quantify impurities within the formulation. The chromatogram obtained from the impurity-spiked formulation solution (illustrated in Figure 10) distinctly displayed peaks corresponding to the investigated impurities. In contrast, the chromatogram from the un-spiked formulation solution exhibited no peaks associated with the studied impurities. This observation indicates that the impurity level in the sample was below the detection limit, confirming that it remained within acceptable limits. This reinforces the successful applicability of the proposed method for precise quantification of process-related impurities in lemborexant.

Conclusion

Forced degradation studies, following the stipulated conditions outlined by the ICH, were conducted on lemborexant. The drug displayed remarkable stability under thermal and UV light degradation conditions, while revealing susceptibility to degradation in acidic, basic, and peroxide environments. The thermal and UV light degradation conditions did not lead to significant degradation. Throughout various stress conditions, a total of six degradation products (DPs) emerged. Among these, DP 1 was consistently identified in both basic and UV light conditions and DP 2 was consistently identified in both base and peroxide. Utilizing LCMS/MS analysis, the characterization of these five distinct DPs, formed during the forced degradation, was effectively achieved, specifically in the ESI positive mode. The mechanisms and pathways underlying the formation of each DP of lemborexant were outlined and discussed. The outcomes of this study hold significant potential in the identification of process-related impurities and potential degradation products, even at trace levels, in bulk drugs. This analytical method stands as a valuable tool for quality control sample assessment, serving regular and stability studies of lemborexant and its associated process-related impurities.

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PROSESLƏ ƏLAQƏLİ LEMBOREKSANT QARIŞIQLARINI QİYMƏTLƏNDİRMƏK VƏ LCMS/MS İLƏ STRESDƏN QAYNAQLANAN PARÇALANMA MƏHSULLARINI AŞKAR ETMƏK ÜÇÜN ETİBARLI HPLC METODUNUN OPTİMALLAŞDIRILMASI

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Bu tədqiqat ilk növbədə proseslə əlaqəli lemboreksant qarışıqlarının səmərəli ayrılması və kəmiyyətcə təyin edilməsi üçün sadə, lakin yüksək həssas HPLC metodunun inkişafına yönəldilmişdir. Bundan əlavə, tədqiqatın məqsədi LCMS istifadə edərək sistematik qiymətləndirmələr yolu ilə lemboreksantın məcburi deqradasiyasının təbiətini öyrənmək idi. Dərman maddəsinin, proseslə əlaqəli qarışıqlar və onun parçalanma məhsullarının (DPs) xromatoqrafik ayrılması Kinetex C18 sütununda (150 x 4,6 mm, 5 mikron) aparıldı, 350 mm ammonium pH 10, asetonitril və metanol format tamponu 6,2:25:10 nisbətində istifadə edərək 35 dərəcə temperaturda saxlanıldı. (v/v) 0,7 ml/dəq sürətlə izokratik elüsyon. aşkarlama dalğa uzunluğu 265 nm olaraq seçildi. Təklif olunan şərtlərdə lemboreksant, sistem üçün məqbul uyğunluğu və spesifikliyi ilə 4.06 və 6.19, 9.33 dəq və 1.60 dəq, müvafiq olaraq 1, 2 və 3 dəq olan qarışıqlar üçün müəyyən edilir. Metod, qarışıqlar üçün 0.009 LOD dəyərini, lemboreksant üçün 40-280 mkq/ml kalibrləmə aralığını və qarışıqlar üçün 0.04 - 0.28 mkq/ml əldə etməyə imkan verir. Qalan doğrulama parametrlərinin həm lemboreksant, həm də qarışıqları üçün icazə verilən dəyərlər daxilində olduğu müəyyən edilmişdir. Qarışıq, ICH q1a (R2) təlimatlarında göstərildiyi kimi müxtəlif stres faktorlarına (turşular, qələvilər, peroksidlər, istilik təsirləri və ultrabənövşəyi radiasiya) məruz qalmışdır. Stress tədqiqatından yaranan çürümə məhsulları müsbət ESI rejimində LCMS/MS ilə aşkar edilmiş və xarakterizə edilmişdir. Bunun üçün parçalanma məhsulları ilə lemboreksant arasında toqquşma nəticəsində yaranan dissosiasiya kütlə spektrometriyası məlumatlarının hərtərəfli müqayisəsi aparılmışdır. Nəticədə, parçalanmaya məruz qalan beş birləşmə üçün mümkün strukturlar təklif edildi. Doğrulama ilə bağlı əlavə tədqiqatların nəticələri eyni dərəcədə qənaətbəxş idi və bu, həm adi dərman, həm də əczaçılıq kompozisiyalarında lemboreksant və əlaqəli qarışıqların müntəzəm kəmiyyət təyin edilməsinə uyğunluğunu təsdiqlədi. Bundan əlavə, bu məlumatlar potensial olaraq lemboreksantın stresdən qaynaqlanan deqradasiya mexanizminə işıq sala bilər.

Açar sözlər: *lemboreksant, proseslə əlaqəli qarışıqlar, HPLC metodunun inkişafı, məcburi parçalanma tədqiqatları, parçalanma məhsullarının identifikasiyası.*