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Research Article

Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Lasmiditan in tablet dosage form

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Abstract

HPLC, in particular, offers high resolution, accuracy, and reproducibility, making it indispensable for routine quality control and advanced research applications. Method development in HPLC involves systematic selection and optimization of mobile phase composition, column chemistry, detection wavelength, and operational parameters to achieve reliable separation and quantification. Analytical method validation, guided by ICH and USP standards, ensures that developed methods meet essential criteria, including accuracy, precision, linearity, specificity, robustness, and sensitivity.

Keywords: Lasmiditan, Development, RP-HPLC, Accuracy, linearity, Limit of detection (LOD) and Limit of quantification (LOQ).

1. INTRODUCTION

Lasmiditan is a selective serotonin receptor agonist classified as a ditan and is used for the acute treatment of migraine attacks. It is available in tablet form at strengths of 50 mg and 100 mg. A review of published analytical methods indicates that only a few procedures are available for estimating Lasmiditan in tablets, and most reported methods lack stability-indicating capability. In particular, earlier methods primarily utilized UV detection, which does not allow assessment of peak purity or confirmation of separation of the drug from its degradation products.[1,2]

In the present work, a specific, accurate, and reproducible stability-indicating RP-HPLC method equipped with a PDA detector was developed for the determination of Lasmiditan in tablet formulation. Chromatographic separation was achieved using a Kromasil C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1 M phosphate buffer (pH adjusted to 3.0) and acetonitrile in a 60:40 v/v ratio. The flow rate was maintained at 1.0 mL/min, and Lasmiditan showed a retention time of 8.303 minutes. Detection at 258 nm provided adequate sensitivity, and the use of a PDA detector ensured peak purity, confirming that the developed method is stability-indicating and suitable for routine quality control.[4,5]

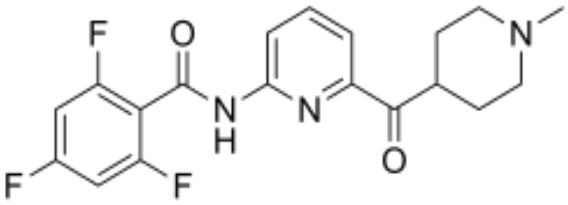

Figure 1: Molecular structure of Lasmiditan

The present study reports a precise, accurate, and robust RP-HPLC method with PDA detection for estimating Lasmiditan in tablet dosage form. The method showed strong linearity, good specificity, and consistent reproducibility across the tested concentration range.[3,6] It successfully separated Lasmiditan from its degradation products, confirming its stability-indicating capability. Compared with earlier methods, this approach offers improved selectivity and better peak purity assessment due to PDA detection. Overall, the developed method is

reliable and suitable for routine quality control and stability analysis of pharmaceutical formulations containing Lasmiditan.[7]

2. MATERIAL AND METHOD

2.1Chemicals and reagents:

The analytical standard compound lasmiditan with purity of 98.35%, its impurity. The tablet formulation containing 50 mg of lasmiditan with brand Abbott Healthcare Pvt. Ltd was purchased from local market. The HPLC grade methanol, acetonitrile, ortho phosphoric acid, were purchased from Merck chemical. The analytical reagent grade chemicals such as, hydrochloric acid (HCl), sodium hydroxide (NaOH), and hydrogen peroxide, Merck chemical.

2.2Instrumentation:

HPLC use model, Shimadzu, Pump: LC-20AD, Detector-SPD-M40, Software: Lab Solution use in development.

2.3Standard stock solution preparation:

Accurately weighed Lasmiditan (25.0 mg) was transferred to a 50.0 mL volumetric flask, dissolved and diluted to the mark with methanol (Concentration- 500 µg/mL).

2.4Selection of mobile phase:

Preparation of Phosphate Buffer pH-3.0:

A phosphate buffer was prepared by dissolving 3.40 g of KH_2PO_4 in 1000 mL of double-distilled water, and the pH was adjusted to 3.0 using orthophosphoric acid. A standard solution of Lasmiditan (8 µg/mL) was prepared by appropriate dilution with the mobile phase and filtered through a 0.22 µm membrane filter prior to injection.

The best separation was achieved using a phosphate buffer (pH 3.0) and acetonitrile mixture (60:40, v/v) at a flow rate of 1.0 mL/min, which produced a sharp and symmetrical Lasmiditan peak with minimal tailing.

2.5 Selection of analytical wavelength:

Aliquot protion of standard stock solution was appropriately diluted with mobile phase to obtain final concentration of 8 µg /mL Lasmiditan. The solution was scanned using a double beam UV-Visible Spectrophotometer-1700 in the spectrum mode between the wavelength ranges of 400 nm to 200 nm against the mobile phase as a blank. The wavelength selected was 258.0 nm as Lasmiditan showed significant absorbance at this wavelength.

2.6 System suitability parameter:

To ascertain reproducibility of the proposed chromatographic system for the estimation of Lasmiditan in tablets, system suitability parameters like tailing factor (T) and column efficiency (number of theoretical plates, N) were studied. Aliquot portion of standard stock solution was appropriately diluted with mobile phase to obtain final concentration of 8.0 µg/mL of Lasmiditan and used for analysis. The solution was filtered through 0.22 µ membrane filter. The filtrate (20 µL) was injected into the column and chromatographed using optimized chromatographic conditions.

2.7 Study of linearity range:

Six linearity test solutions for Lasmiditan were prepared as follows: From the standard stock solution, 5.0 mL was transferred to a 25.0 mL volumetric flask and diluted to the mark with mobile phase. Further, 1.0, 2.0, 3.0, 4.0 and 5.0 mL of the above solution were transferred individually to 10.0 mL volumetric flasks and diluted to the mark with mobile phase, giving concentrations of 2.0, 4.0, 6.0, 8.0, and 10.0 µg/mL of Lasmiditan.

2.8 System Precision:

Preparation of standard solution: From standard stock solution 3.0 ml solution was diluted to 25.0 mL with mobile phase (concentration-60 µg/mL). Further diluted 1.0 mL of above solution to 10.0 mL with mobile phase (Concentration- 6 µg/mL). The solution was then filtered through 0.22 µ membrane filter. Six replicate injections of the diluted solution were chromatographed under optimized chromatographic conditions and the peak area was recorded. The percent relative standard deviation for peak area of six replicates was calculated.

2.9Analysis of Marketed Formulation:

Preparation of Standard Solution: Accurately weighed quantity (25 mg) of Lasmiditan was transferred to a 50.0 mL volumetric flask, dissolved and diluted to the mark with methanol. The solution was ultrasonicated for 5 min and filtered through Whatman filter paper no. 42. From the filtrate, 3.0 mL solution was diluted up to 25.0 mL with mobile phase. From the above solution, 1.0 mL was diluted to 10.0 mL with the mobile phase. The diluted solution was filtered through 0.22 µ membrane filter.

3.0 Preparation of sample solution:

Twenty tablets were weighed, and the average weight was calculated. The tablets were then triturated to obtain fine powder. Accurately weighed quantity of tablet powder equivalent to about 25 mg of Lasmiditan was transferred to 50.0 mL volumetric flask, added 30 mL of methanol and the contents of flask were sonicated for 5 minutes, the volume was then made up to the mark and the flask was shaken for two minutes. The solution was filtered through whatmann filter paper no. 42. From the filtrate, 3.0 mL of solution was diluted up to 25.0 mL with mobile phase. From the above solution, 1.0 mL solution was diluted up to 10.0 mL will mobile phase. The diluted solution was filtered through 0.22 µ membrane filter. Equal volume of standard and sample solution (20µl) were injected in to the system and chromatographed in triplicate using optimized chromatographic conditions. The corresponding chromatogram were recorded at 258 nm.

3. METHOD VALIDATION:

The proposed method was validated by studying several parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

3.1 Preparation of Sample Solution:

Accurately weighed quantity of pre-analysed tablet powder equivalent to about 25 mg Lasmiditan was transferred individually into six different 50.0 mL volumetric flasks. To each of the flasks, the following quantities were added,

Level of Recovery 80%: Flask No. 1- 20 mg Lasmiditan

Level of Recovery 80%: Flask No. 2- 20 mg Lasmiditan

Level of Recovery 100%: Flask No. 3- 25 mg Lasmiditan

Level of Recovery 100%: Flask No. 4- 25 mg Lasmiditan

Level of Recovery 120%: Flask No. 5- 30 mg Lasmiditan

Level of Recovery 120%: Flask No. 6- 30 mg Lasmiditan

3.2 Intra-day Precision:

Intraday precision was determined by analyzing the tablet formulation as described under analysis of marketed formulation at different time intervals on the same day. B. Inter-day Precision: Inter-day precision was determined by analyzing the tablet sample as described under analysis of marketed formulation on three different days.

3.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Calibration curves for solution plotted by taking the peak area verses concentration. The LOD and LOQ for determined using calibration curve. LOD and LOQ were calculated as3.3s/S and 10s/S. where the standard deviation of y- intercept of regression equation and S is the slope of the calibration curve (n=3).

3.4 Robustness:

Robustness of the proposed method was studied by small but deliberate variations in the optimized method parameters. The effect of changes in flow rate, pH and mobile phase composition on retention time and tailing factor were examined.

3.5 Forced degradation studies:

Accurately weighed quantity of tablet powder equivalent to about 25 mg of lasmiditan was transferred separately to six different 50.0 mL volumetric flask, (flask no. 1, 2, 3, 4, 5 and 6). To flask no. 1, 2 and 3, added 3.0 mL methanol as co-solvent followed by addition of 3.0 mL 0.1M HCl, 0.1 M NaOH and 3 % H_2O_2, respectively. For neutral hydrolysis, 3.0 mL of water was added to flask no.4. The contents of flask no. 1, 2, 3 and 4 were heated on water bath at 80^0C for 3 hr. Flask no. 5 containing tablet powder was kept in hot air oven at 80^0C for 24 hr to study the effect of heat on tablet sample (heat degradation).

4. RESULT AND DISCUSSION

4.1 Optimum Chromatographic Conditions:

HPLC Column: Kromasil C18, (250 mm X 4.6 mm, 5µm)

Column temperature: Ambient temperature

Mobile Phase: Phosphate Buffer (pH 3.0) : Acetonitrile (60 : 40 v/v)

Flow rate: 1.0 mL/min

UV detection: 258.0 nm

Injection volume: 20 µL

Run time: 10 min

The typical of Lasmiditan obtained under optimized chromatographic conditions is as follows,

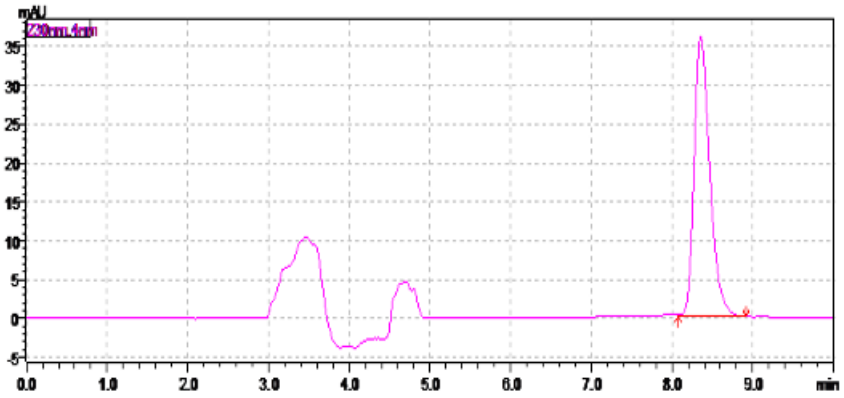

Figure 2: Typical chromatogram of Lasmiditan (Rt = 8.356 min)

To ascertain the reproducibility of the above chromatographic conditions, system suitability tests were carried out. Results of system suitability parameters are as follows,

Table 1: System suitability parameters

Parameter	Observes Values
Tailing Factor	1.255
HETP	17.289

The tailing factor and the HETP values are acceptable as per the pharmacopeial standards.

4.2 Study of Linearity Range:

The linearity of detector response was studied in the range of 2-10 µg/mL of Lasmiditan. The mean peak area obtained and the standard calibration curve obtained in the concentration range under study is as follows,

Table 2: Standard calibration data of Lasmiditan

Sr. No	Concentration (µg/ml)	Peak Area
1	2.0	173453
2	4.0	329126
3	6.0	484560
4	8.0	644812
5	10.0	807296

*Mean of three determinations

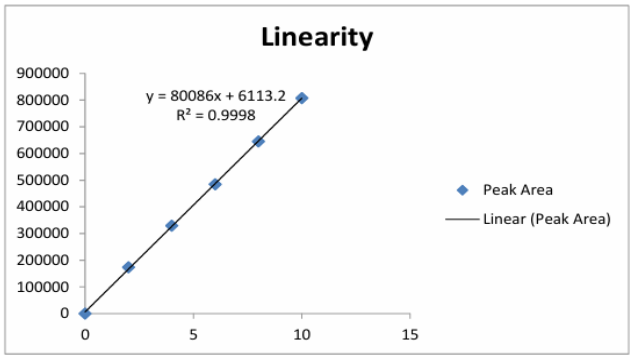

Figure 3: Standard calibration curve for Lasmiditan

The linear detector response was observed in the concentration range of 2-10 µg/mL with the co-relation coefficient of 0.9998.

4.3 Study of System Precision:

The mean peak area obtained for six replicate injections of Lasmiditan (Concentration- 6 µg/mL) chromatographed under optimized chromatographic conditions and the percent relative standard deviation obtained is as follows,

Table 3: System Precision Study

Replicate No	Concentration (µg/ml)	Peak area*
1	6.0	484427
2	6.0	487135
3	6.0	490371
4	6.0	484289
5	6.0	483976
% RSD		0.563%

The percent relative standard deviation was found to be less than 2.0 indicating the system precision.

Analysis of Marketed Formulation:

Table 4: Statistical Validation of Analysis of Tablet Formulation

Sr. no	Drug	Amount of Drug Estimated (mg/tablet)	% Label Claim	SD	% RSD
1	Lasmiditan	49.88	99.74	1.045	1.08%

Accuracy Studies:

The results of percent recovery estimated by proposed HPLC method at three different levels viz. 80 %, 100 % and 120 % using standard addition method are as follows,

Table 5: Result of Accuracy Studies

Percent Level of Recovery	Weight of Tablet Sample (g)	Amount of Pure Drug Added (mg)	Total Amount of Drug Estimated in Sample (mg)	Amount of Pure Drug Recovered (mg)	Percent Recovery
80	0.0563	20.3	45.49	20.14	99.23
80	0.0558	20.5	45.36	20.22	98.61
100	0.0549	25.1	49.81	25.10	100.02
120	0.0556	30.2	54.93	29.90	98.99
120	0.0571	30.2	55.54	29.83	98.77

Table No 6: Statistical Evaluation of Recovery Studies

Sr. No	Drug	Mean Percent Recovery	SD	%RSD
1	Lasmiditan	99.03	0.492	0.545%

The per cent recovery was found in the range of 98.59 % to 100.02 % with an average per cent recovery of 99.03 % indicating the accuracy of the proposed RP-HPLC method.

4.4Precision Study:

The results of the intra-day precision and inter-day precision study of the proposed HPLC method are as follows,

Table 7: Results of the Precision study

Sr. No	Precision	Mean Percent Label Claim	SD	%RSD
1	Intra-Day Precision	99.45	1.516	1.524
2	Inter-Day Precision	100.30	1.428	1.423

*mean of three determinations.

The standard deviation and per cent relative standard deviation for the intra-day and inter-day precision study were found to be less than 2.0, indicating the precision of the proposed RP-HPLC method.

4.5Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD and LOQ were calculated as 3.3s/S and 10s/S. where the standard deviation of y- intercept of regression equation and S is the slope of the calibration curve (n=3). The results obtained are as presented in the following table. The low values of Limit of Detection and Limit of Quantitation indicate the sensitivity of the proposed RP-HPLC method.

Table 8: Results of LOD and LOQ

Parameter	Lasmiditan
Limit of Detection (µg/mL)	0.033
Limit of Detection (µg/mL)	0.103

4.6Robustness:

The results of robustness of the proposed method studied by small but deliberate variations in the optimized method parameters such as changes in flow rate, pH and mobile phase composition and their effect on retention time and tailing factor are as follows,

Table 9: Result of Robustness Studies

Sr. No	Factor	Level	Retention Time	Tailing factor
1	**Change in Flow Rate**	0.9	9.240	1.418
		1.0	8.165	1.255
		1.1	7.535	1.347
	SD			
2	**Change in pH**	pH-2.9	8.342	1.283
		pH-3.0	8.119	1.251
		pH-3.1	8.277	1.310
	SD			
3	**Change in Mobile Phase Composition**	59:41	8.437	1.432
		60:40	8.437	1.121
		61:93	8.248	1.296
	SD			

The change in method parameters did not affect the retention time and tailing factor for Lasmiditan peak indicating the robustness of the proposed RP-HPLC method.

4.7 Forced Degradation Study:

In forced degradation studies, intentional degradation was tried by exposing tablet powder sample to following stress condition, acidic (0.1 M HCl), alkaline (0.1 M NaOH), oxidation (3 % H2O2), neutral hydrolysis, Heat (800C for 24 hrs) and UV exposure (for 24 hrs). For acidic condition content in flask were kept in water bath for 6 hr. and for alkaline, oxide and neutral conditions the content in the flasks were kept in water bath at 800C for 2 hr. After the respective time intervals all the flasks were removed and allowed to cool. The samples were then analyzed in similar manner as described under analysis of tablet formulation. The chromatograms obtained for samples treated with different stress conditions and the results of forced (stress) degradation studies are as follows,

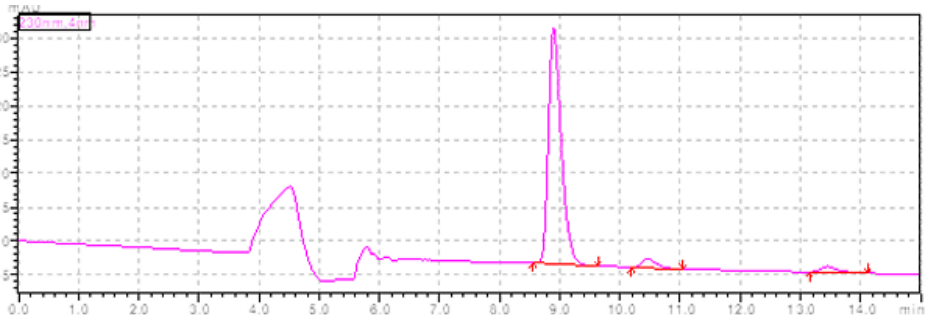

Figure 4: Chromatogram of alkali (0.1 M HCl) treated sample

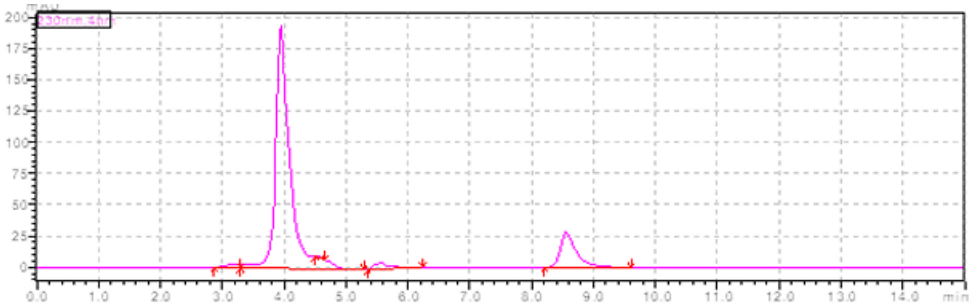

Figure 5: Chromatogram of acid (0.1M NaOH) treated sample

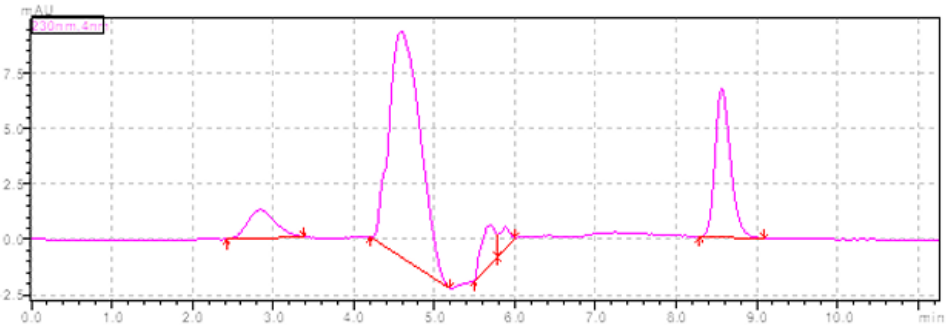

Figure 6: Chromatogram of oxide (3% H_2O_2) treated sample

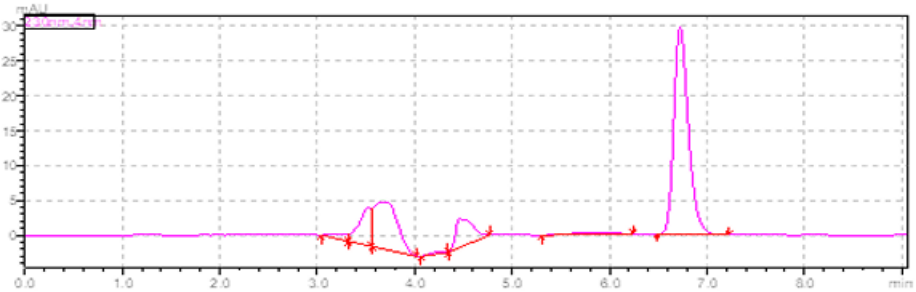

Figure 7: Chromatogram of the sample exposed to neutral hydrolysis (H_2O)

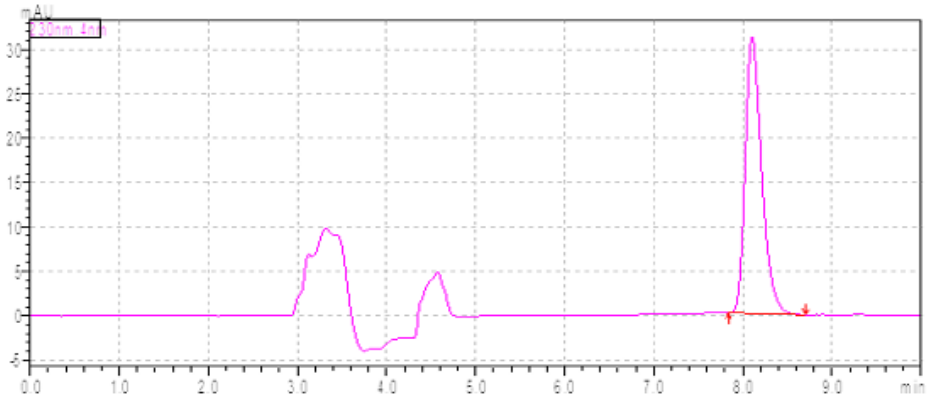

Figure 8: Chromatogram of heat treated sample for 24 hrs

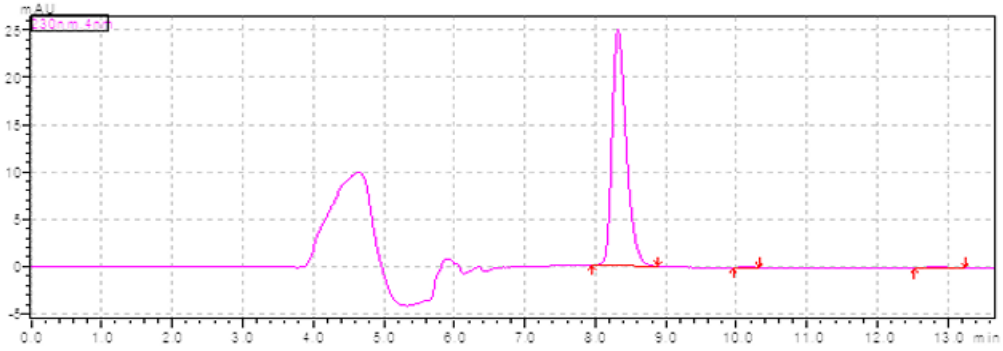

Figure 9: Chromatogram of photo-degraded (UV) sample for 24 hrs.

Table 10: Results of Forced Degradation Studies

Sr. No	Stress Condition	Temperature and Time	% assay of active substance	Retention Time of degraded peaks
1	Acid (0.1 HCl)	80^0C for 3 hrs	96.66	10.47
2	Alkali (0.1M NaOH)	80^0C for 3 hrs	33.31	04.06
3	Oxide (3% H_2O_2)	80^0C for 3 hrs	72.83	2.94
4	Neutral (H_2O)	80^0C for 3 hrs	99.15	--
5	Photo Degradation	UV Light 24 hrs	98.05	--
6	Therma;	60^0C for 24 hrs	98.97	--

CONCLUSION:

A reliable and efficient RP-HPLC method using a PDA detector was developed and validated for the estimation of Lasmiditan in tablet formulations. The method demonstrated good linearity, precision, accuracy, and robustness within the tested concentration range. It showed high specificity and successfully quantified Lasmiditan in the presence of degradation products, confirming its stability-indicating nature. The use of PDA detection enabled practical evaluation of peak purity and improved analytical confidence compared to earlier methods. Owing to its reproducibility and selectivity, the proposed method is suitable for routine quality control and stability studies of Lasmiditan tablets.

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Ethics approval: Not Applicable.

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