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Development of a stability-indicating HPLC method for Lasmiditan and its process related impurities with characterization of degradation products by LC-MS/MS

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Abstract: The present investigation aimed to investigate a novel approach involving the utility of liquid chromatography (LC) and liquid chromatography-mass spectrometry (LC–MS) for separation and characterization of very nominal quantities of degradation products (DPs) of lasmiditan. The process related impurities along with lasmiditan were resolved on Inertsil ODS-3 (250×4.6 mm, 5.0 µm) column at room temperature using 0.1 M phosphate buffer with pH 3.6 and acetonitrile 65:35 (v/v) as mobile phase A, 0.1 M phosphate buffer with pH 3.6 and methanol in the ratio of 20:80 (v/v) as mobile phase B. The mobile phase solvent A and B were mixed at 50:50 (v/v) and the mixture was pumped isocratically at 1.0 mL/min and UV detection at 246 nm. The method shows sensitive detection limit of 0.003 µg/mL for impurity 1, 0.008 µg/mL, 0.005 µg/mL for impurity 4, 0.008 µg/mL for impurity 1 and 2 with calibration curve liner in the range of 25-150 µg/mL and 0.025-0.15 respectively for lasmiditan and its studied impurities. The validation including system suitability, specificity, accuracy, recovery, ruggedness and robustness were noticed to be acceptable. The lasmiditan pure compound was subjected to stress studies as per guidelines of International Conference on Harmonization (ICH). The stress study results suggest that, lasmiditan was labile in acid, base and UV light conditions, whereas stable in thermal and peroxide conditions. A total of five degradation products (DPs) were formed and was characterized using fragmentation pattern as well as masses obtained on LC–MS/MS. The hitherto unknown DPs were identified 2,4,6-trifluoro-N-{6-[hydroxy(2,3,4,5-tetrahydropyridin-4-yl)methyl]pyridin-2-l}benzenecarboximidic acid (DP 1), N-[6-(2-ethylbutanoyl)pyridin-2-yl]-2,4,6-trifluorobenzamide (DP 2), 2,4,6-trifluorobenzamide (DP 3), 2,3-dihydropyridin-4-yl(pyridin-2-yl)methanol (DP 4) and 2,4,6-trifluoro-N-[6-(2-methylidenebutyl) pyridin-2-yl]benzamide (DP 5). The proposed method was successfully applicable for routine analysis of lasmiditan and its process related impurities in pure drug and formulations and also applicable for identification of known and unknown impurities of lasmiditan.

Keywords: Lasmiditan; impurities; HPLC method; stress degradation studies; LCMS characterization; degradation products.

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1. Introduction

The pharmaceutical industry is expanding daily with the goal of researching novel pharmaceuticals that are derived from natural materials or chemically produced. The key issue with that is that the product needs to be as pure as possible, and purity was thought to be a crucial component in maintaining the quality of the drugs [1]. It is possible for certain undesirable chemicals to persist in the pure medicine after its manufacture, and these undesirable compounds were referred to as impurities. Even extremely small amounts of these contaminants can have an impact on the drug product's quality, effectiveness, and safety [2]. As a result, it was believed that impurity identification and quantification were crucial for generating safe drugs, and that HPLC was an easy and practical method for doing so from any source.

Lasmiditan is a medical drug belongs to selective serotonin receptor agonists class and was used for the treatment of active but short-term migraine with or without aura in adults [3]. Lasmiditan was used as monotherapy in treating acute migraine and also suggested as an abortive therapy in adults patients who in treatment with chronic migraine [4]. It was also considered as an option for patients who doesn't relief with triptans or other migraine treating agents or who were not able to use other migraine treating agents due to contraindications [5]. The most common adverse effect associated during the use of lasmiditan includes paresthesia, fatigue, dizziness, drowsiness, muscle weakness and nausea. The adverse symptoms such as speech abnormalities, cognitive changes and hallucinations were observed in less than 2 % of study participants [6]. The symptoms like hallucinations consistent with serotonin syndrome, speech abnormalities and cognitive changes were identified in less than 2 % of studied participants [6]. Figure 1 presents the molecular structure of lasmiditan.

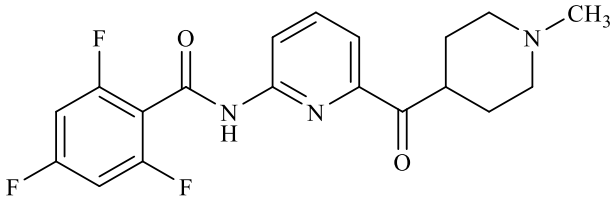
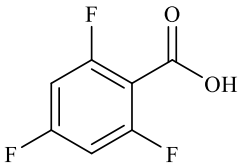


Figure 1. Molecular structure of Lasmiditan

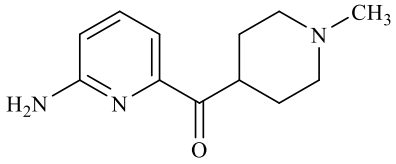
There are different analytical methods reported for estimation of lasmiditan in pharmaceutical formulations using HPLC [7-9]. The elaborative literature review suggests that, no method available for resolution, identification and quantification of process related impurities of lasmiditan. Hence this study intended to fulfill the gaps identified in literature. Based on availability, the process related impurity 1, 2, 3 and 4 were utilized in the study. The structure and properties of process related impurities of lasmiditan was presented in Figure 1.

The synthesis route of lasmiditan pure drug [10] was verified for the evaluation of the route of formuation of process related impurities in the final product. The 2,4,6-trifluorobenzoic acid which was utilized as starting product (**IV**) in the synthesis process was remained in the final product as undesired compound and was designated as impurity 1. The impurity 2 and 4 was originated during the formation of lasmiditan (**I)** due to the side reaction of 2,4,6-trifluoro-N-(pyridin-2-yl)benzamide (**XII)** and N-methoxy-N,1-dimethylpiperidine-4-carboxamide (**III)**. The impurity 3 is a byproduct formed due to the reaction of impurity A (**IV**) with formed lasmiditan (**I**). These impurities remains in the final product as impurities and that will affect the quality and purity of the final product. The mechanism of synthesis of lasmiditan was presented in the supporting information file.

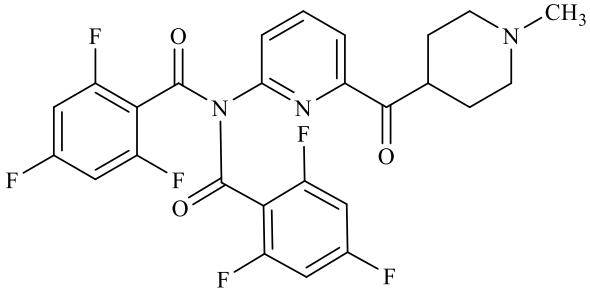


Impurity 1

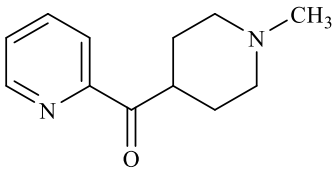
2,4,6-trifluorobenzoic acid



Impurity 2
(6-aminopyridin-2-yl)(1-methylpiperidin-4-yl)methanone



Impurity 3
2,4,6-trifluoro-N-(6-(1-methylpiperidine-4-carbonyl)pyridin-2-yl)-N-(2,4,6-trifluorobenzoyl)benzamide



Impurity 4
(1-methylpiperidin-4-yl)(pyridin-2-yl)methanone

Figure 2. Molecular structure of process related impurities of Lasmiditan

2. Materials and Methods

2.1. Chemicals and Reagents

The analytical standard compound lasmiditan with purity of 98.35%, its impurity 1, 2, 3 and 4 were procured from Eli Lilly & Co (India) Pvt. Ltd, Gurgaon, Haryana. The tablet formulation containing 100 mg of lasmiditan with brand Reyvow® was purchased from local market. The HPLC grade methanol, acetonitrile, Milli-Q® water were purchased from Merck chemicals, Mumbai. The analytical reagent grade chemicals such as acetic acid, sodium acetate, hydrochloric acid (HCl), sodium hydroxide (NaOH), and hydrogen peroxide were purchased from Fisher scientific, Mumbai.

2.2. Instrumentation

The HPLC analysis was performed on Agilent 1100 (USA) instrument that coupled with quaternary pump (G1311 A) for solvent delivery. The analytes were injected through temperature adjustable auto sampler (G 1329A) having injection capacity of 0.1–1500 µL. The column eluents were detected using programmable ultraviolet (UV) detector (G 1314 A) and the chromatographic integrations were carried using Agilent chem-station software. The LCMS analysis was performed on Waters LCMS (Japan) equipped with triple quadruple mass detector and MassLynx software.

2.3. Standard Solution Preparation

The standard lasmiditan and its impurities at 1 mg/mL (1000 µg/mL) were prepared separately by accurately weighing 25 mg of analyte in 25 mL of volumetric flask containing 15 mL of methanol. The analytes were dissolved in solvent using an ultrasonic bath sonicator. Then the analytes were filtered through 0.2 µm membrane filter and the final volume was made up to the mark using same solvent to obtain 1000 µg/mL concentration of lasmiditan and its impurities separately. During the analysis, selected volume of required concentration of individual analyte was mixed separately.

2.4. Test Solution Preparation

The Reyvow® tablets with 100 mg strength were used for the preparation of formulation solution. The tablets were fine powdered using clean, dry mortar - pestle and the tablet powder equivalent to 25 mg of lasmiditan was weighed accurately. The weighed tablet powder was taken in a 25 mL volumetric flask containing 15 mL methanol. Then the drug was dissolved completely in solvent and the final volume made up to the mark. Then, it was filtered and was diluted to standard concentration and the dilute solution was used for evaluation of formulation assay.

2.5. Method Development

The UV-visible spectrophotometer technique was utilized for evaluating appropriate wavelength for detection of lasmiditan along with its impurities. The lasmiditan and its impurities solution at 10 µg/mL concentration was scanned using spectrophotometer separately and the overly absorption spectra confirm the suitable wavelength. Various configurations of stationary phases with different manufactures were varied for the best resolution of lasmiditan and its impurities. The mobile phase was optimized by change in different solvent compositions with different pH ranges and different flow rates were varied. The conditions that resolve the analytes was further studied for validation.

2.6. Method Validation

The method developed in the study was validated for its acceptable performance to ensure suitability of indent purpose. The parameters such as range, linearity, accuracy, precision, specificity, detection limit, quantification limit, ruggedness and robustness experiments were executed as per reported literature [11-14] and ICH guidelines [15].

The limit of quantification (LOQ) and limit of detection (LOD) for impurities were evaluated by determining signal to noise ratio of 10:1 and 3:1, respectively, by analysing progressively known concentrations of impurity solutions in the optimized HPLC method. The precision experiment was also conducted at LOQ level by analysing six independent preparations of impurities and the %RSD (relative standard deviation) of areas response observed for each impurity was determined.

The method linearity was evaluated by injecting mixed calibration dilutions at six concentrations ranging from LOQ to 150% of specification limit. The least squares linear regression method was adopted for plotting linear regression equations for lasmiditan and its impurities separately.

The method precision was investigated by analysing six individual preparations in the same day for intraday precision, three different days by same analyst for interday precision and six times by six different analysts in the same day in ruggedness. The method robustness was verified by introducing minor variations in the developed method parameters, including the mobile phase mixture, pH, detector wavelength and column temperature. The selectivity, resolution between each nearby peak and the % change in the peak area response were evaluated in each of the altered condition. The method accuracy was evaluated by spiking in triplicate to a pre-analysed sample at four different concentration levels such as LOQ, 50%, 100 % and 150%. The % recovery in each analysis and the % RSD of recovery in each spiked level was summarized for evaluating the method accuracy.

The specificity and stability indicating power of the developed method was evaluated by performing stress degradation study. Various stress studies like acid, base, peroxide, thermal and

ultraviolet stress studies were performed for standard lasmiditan. The placebo, lasmiditan pure drug and tablet powder were exposed 5 mL of hydrochloric acid (1 N), 5 mL of sodium hydroxide (1 N) and 5 mL of hydrogen peroxide (3.0 %) separately for acid, base and peroxide degradation study respectively for 12 hours. Then, the stress exposed samples were neutralized with suitable diluent. The samples were kept in a photolytic chamber irradiated with UV light at 1.2 million Lux/h and 103,959 Wh/m^2 for UV light degradation study and samples were kept in an air oven at 80 °C for 24 hours for thermal degradation study. The all the stress exposed samples were diluted to target concentration and then the diluted samples were analysed in the developed method. The resultant chromatograms and chromatographic response was utilized for evaluating the stability indicating nature of the proposed method.

The stress degradation compounds (DPs) were characterized using mass spectral analysis. The mass spectral analysis was performed by allowing 40 % of the column eluents in to the column using a splitter and the samples were recorded using mass detector that was operated in ESI (Electrospray ionization) positive ion mode. The mass detector was operated with adequate conditions such as capillary voltage (3200-3600 V), fragmentor voltage (70 V) and skimmer voltage (60 V). Nitrogen gas was utilized for drying (300 ^{o}C, 9 L/h) and nebulization (40 Psi). The spectra throughout the analysis were recorded under similar experimental conditions and 20-30 average scans were conducted.

The proposed method was applied for identification and quantification of process related impurities of lasmiditan in formulations. The Reyvow® formulation solution spiked with known concentration of impurities and the un-spiked solution was analysed in the proposed method. The % assay of impurities was calculated by comparing peak area response of each impurity in its corresponding calibration curve.

2.7 Assessment of Uncertainty

In the process of method validation, the uncertainty contributions were measured as per EURACHEM guide. The uncertainty related to purity of compounds ($u_{standard\ purity}$), sample weighing ($U_{weighing}$) calibration results such as slope ($u_{calibration}$), recovery ($u_{recovery}$) and precision ($u_{repeatability}$) was evaluated as main uncertainty contributions. The overall combined uncertainty ($u_{combined}$) was evaluated using formula:

$$U_{combined} = \sqrt{u_{standard\ purity)}{}^2 + (u_{calibration)}{}^2 + (u_{recovery)}{}^2 + (u_{repeatability)}{}^2}$$

The overall expanded uncertainty ($U_{expanded}$) was determined at 95% confidence level by multiplying the achieved combined uncertainty with coverage factor (k) equal to 2. The EURACHEM Guide [16] and reported literature [17] was utilized for calculating the uncertainty.

3. Results and Discussions

The impurity profiling was performed to identify known and unknown impurities present in a pure drug will helpful for producing safe pharmaceutical product. The literature survey reveals that there is no analytical method available for quantification of process related impurities of lasmiditan and hence this study aimed to develop a simple HPLC method for identification and quantification of process related impurities 1, 2, 3 and 4 of lasmiditan.

The impurities 1, 2, 3 and 4 along with standard lasmiditan were polar compounds and hence method optimization was performed on non-polar stationary phase for best retention of analytes. During the selection of column, lasmiditan standard and its impurities were co-eluted with columns of different configurations like Octadecylsilane, cyano, phenyl–hexyl and amino columns with different length and particle size. Different composition of mobile phases including buffers such as acetate and phosphate with pH range of 2-6 with methanol and acetonitrile as organic modifier were studied. The ionizable

hydrophilic functional groups like –COOH and –NH etc., were expected to resolve using mobile phase with pH buffers and hence the effect of buffer pH on the separation and retention of impurities was studied in the pH range of 2-7. At pH 2.0 to 2.5, the impurity 2 was co-eluted with impurity 4 whereas at pH 5.0 to 5.5 the impurity 1 was co-eluted with impurity 2 and hence these pH ranges was not considered for further optimization. Good resolution of these analytes were obtained with the use of monobasic potassium phosphate buffer in the pH range of 3.5 to 4.0 and hence further trails were performed using monobasic potassium phosphate buffer in the mobile phase. Successful separation of lasmiditan and its impurities was achieved using Inertsil ODS-3 (250×4.6 mm, 5.0 µm) column at room temperature was selected as stationary phase for best resolution and peak symmetry.

Successful separation with better selectivity and resolution of analytes was attained using 0.1 M phosphate buffer with pH 3.6 and acetonitrile 65:35 (v/v) as mobile phase A, 0.1 M phosphate buffer with pH 3.6 and methanol in the ratio of 20:80 (v/v) as mobile phase B. The mobile phase solvent A and B were mixed at 50:50 (v/v) and the mixture was pumped isocratically at 1.0 mL/min. The column temperature was kept at 35 ^{o}C, sample injection volume of 20 µL and detection wavelength of 246 nm was kept constant throughout the analysis.

In this condition, the shape of the peaks corresponds to lasmiditan and its impurities were noticed to be symmetric and the resolution between the nearby peaks was observed to be more than 2. The compounds in the standard solution were identified by injecting the individual standard solution and comparing the individual retention time with standard. The retention time of the analytes was noticed to be 8.49 min for lasmiditan, 7.24 for impurity 1, 5.58 min for impurity 2, 10.54 for impurity 3 and 4.90 for impurity 4. The column efficiency for lasmiditan and its all impurities was noticed to be lesser than the limit in case of tail factor and greater than acceptable limit in case of theoretical plates and resolution and results were illustrated in Table 1. Placebo and the standard lasmiditan solution spiked with impurities chromatogram were presented in Figure 3 and based on chromatograms it was confirmed that the method was specific for the separation and detection of process related impurities of lasmiditan.

In each method validation run, the system suitability of peaks corresponds to lasmiditan and its impurities were verified and the acceptance criteria in each validation run were theoretical plates (N) of > 2000, resolution (R_S) between adjacent peaks was > 2 and tailing factor (A_S) of ≤ 2.0 [15]. The N, R_S, and A_S of peaks observed for lasmiditan and its impurities were within the acceptable limits (Table 1), so the optimized method is suitable for analysis of these compounds.

The sensitivity of the method for the detection of impurities was identified by determining the LOD and LOQ of impurities in the developed method which was performed by adoption s/n ratio method. Based in the results observed, the LOD was confirmed as 0.003 µg/mL, 0.008 µg/mL, 0.008 µg/mL and 0.005 µg/mL respectively for impurity 1, 2, 3 and 4 proved that the method was very sensitive and can detect the analytes at very low concentrations. The sensitive results were presented in Table 1.

The calibration curve dilutions of the impurities were prepared based on the sensitive results of the impurities and the quantification limit was considered minimum concentration in calibration range. The concentration of lasmiditan was considered such that the solution contains 0.1 % each impurity. Two calibration curve ranges with six points each were obtained separately for lasmiditan and its impurities by plotting the analyte peak area (A) against analyte strength. The equations of the calibration curves were determined using linear least squares regression analysis and results were presented in Table 1. The calibration curve was linear in the concentration level of 25-150 µg/mL and 0.025-0.15 respectively for lasmiditan and its studied impurities. These results suggest that the method show good linearity that could be applicable to quantify impurities at very nominal concentrations.

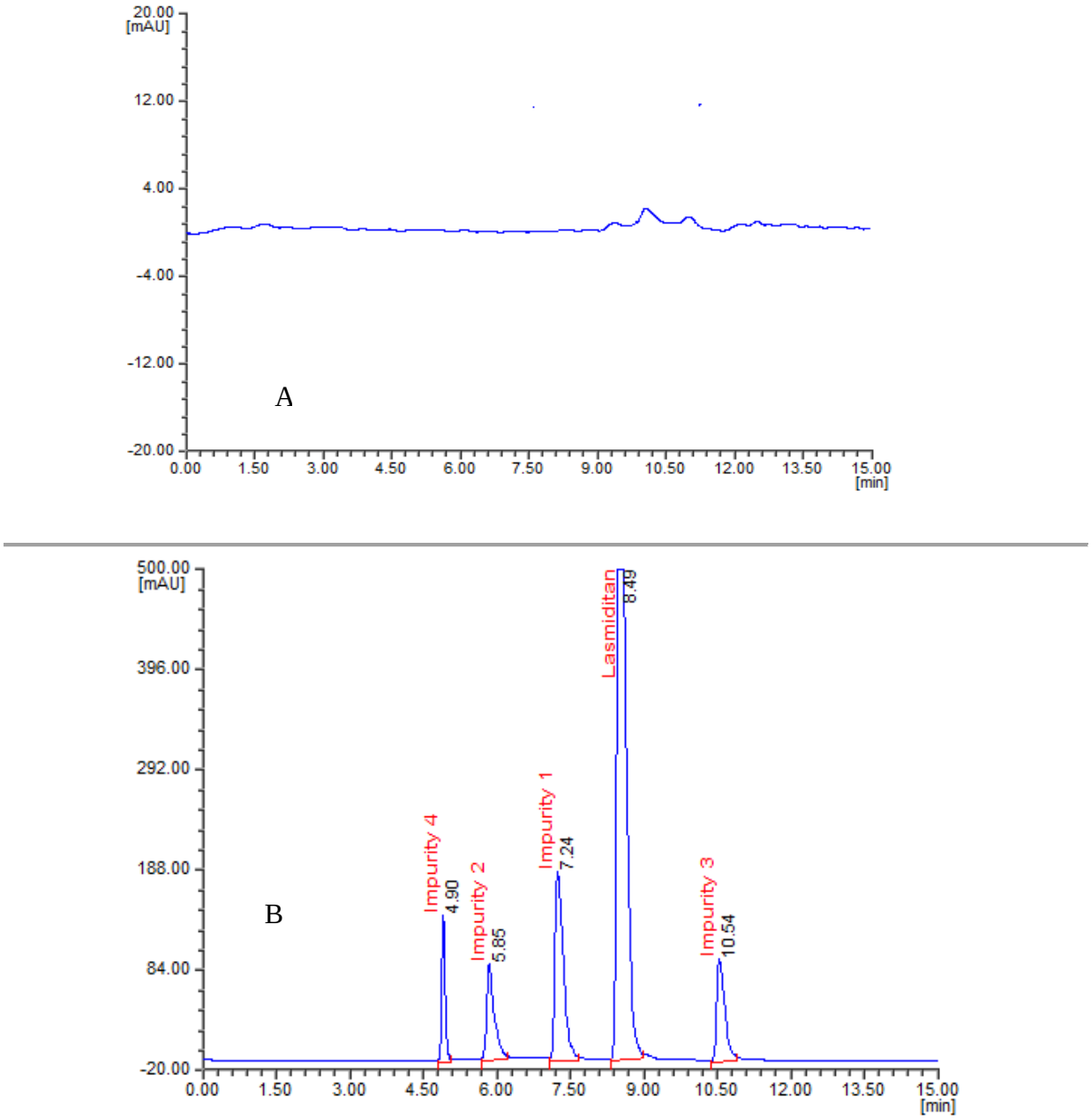


Figure 3. Specificity chromatograms of lasmiditan and its impurities in the proposed method
A) placebo sample chromatogram obtained in the proposed method; **B)** chromatogram obtained for analysing the standard solution of lasmiditan at at 100 µg/mL concentration spiked with 0.1 % impurities in the developed method

The method precision and reproducibility was performed as intra, interday precision and ruggedness experiments. The % RSD of lasmiditan and its impurities was calculated to be less with in the acceptable limit of less than 2 (Table 1) proves that the method was precise and rugged for the analysis of lasmiditan and its impurities. The methos accuracy was based on recovery (R %) of known quantity of analytes in placebo and was calculated by using the formula:

$$R\% = C_{found} \times 100 / C_{taken}$$

The method accuracy was investigated by performing three replicate injections of samples having 50 µg/mL, 100 µg/mL and 150 µg/mL for lasmiditan spiked with 0.1 % of studied impurities. The acceptable %RSD of less than 2 and % recovery of 98-102 was noticed for lasmiditan and

impurities studied suggest the method was accurate. The summary results observed in system suitability, linearity, precision, accuracy and sensitivity study in the proposed method were presented in Table 1.

Table 1. Method validation results observed in the proposed method

Parameter	Results				
	Lasmiditan	**Impurity 1**	**Impurity 2**	**Impurity 3**	**Impurity 4**
System suitability[$]					
t_R (min)	8.49	7.24	5.85	10.54	4.90
RRT	--	0.85	0.68	1.24	0.58
RRF	--	0.09	0.04	0.06	0.07
R_S	5.87	6.93	5.21	9.36	--
A_S	1.06	1.03	1.07	1.01	0.95
N	10241	8591	5782	14857	4920
Linearity					
Range (µg/mL)	25-150	0.025-0.15	0.025-0.15	0.025-0.15	0.025-0.15
Slope	8166.6	706988	348073	461148	567104
Intercept	5127.2	409.7	186.23	1072.7	460.79
r^2	0.999	0.9996	0.9992	0.999	0.9991
Precision[$$]					
Intraday	0.22	0.25	0.74	0.77	0.30
Interday (day 1)	0.82	0.69	0.47	0.76	0.54
Interday (day 2)	1.43	1.15	0.23	0.38	0.18
LOQ level	--	0.10	0.21	0.17	0.64
Accuracy at 50 % level[$]					
Prepared (µg/mL)	50	0.05	0.05	0.05	0.05
Recovered (µg/mL)	49.252	0.0495	0.0493	0.0497	0.049
% Recovery	98.50	98.99	98.61	99.30	98.540
% RSD	0.23	0.55	0.339	0.64	0.34
Accuracy at 100 % level[$]					
Prepared (µg/mL)	100	0.10	0.10	0.10	0.10
Recovered (µg/mL)	99.023	0.0990	0.0993	0.0988	0.0996
% Recovery	99.02	98.9900	99.32	98.82	99.6333
% RSD	0.87	0.62	0.678	0.39	1.21
Accuracy at 50 % level[$]					
Prepared (µg/mL)	150	0.15	0.15	0.15	0.15
Recovered (µg/mL)	148.205	0.1488	0.1492	0.1508	0.1496
% Recovery	98.80	99.20	99.44	100.56	99.71
% RSD	0.79	0.74	0.83	0.29	0.81
Sensitivity					
LOD (µg/mL)	--	0.003	0.008	0.008	0.005
LOQ (µg/mL)	--	0.010	0.025	0.025	0.015

t_R (min) = retention time; RRT = relative retention time; RRF = relative response factor; R_S = resolution; A_S = tail factor; N = No. of theoretical plates; r^2 = slope; [$]average of three determinations; [$$]average of six determinations

The method robustness evaluated by making deliberate small modifications in the proposed method conditions and the effectiveness of the method for the resolution and quantification of lasmiditan and its impurities was assessed. In this study the mobile phase composition was changed as

45:55 (v/v) and 55:45 (v/v) of mobile phase A and B in MP 1 and MP 2 respectively. The pH of the mobile phase was altered as 3.5 in pH 1, 3.7 in pH 2, detector wavelength altered as 241 nm in WL 1 and 251 nm in WL 2 whereas the column temperature was altered as 30 ºC in CT 1 and 40 ºC in CT 2 respectively. In all the altered conditions, the % change in the peak area response and the system suitability of lasmiditan and its impurities were summarized in Table 2. In all the altered conditions, no prominent changes were observed proved that the method was robust.

Table 2. Robustness study results in the proposed method

S No	Changed condition	Parameter	Results observed				
			LDT	Imp 1	Imp 2	Imp 3	Imp 4
1	MP 1	% change	0.99	0.32	0.61	0.73	1.45
		t_R	8.51	7.23	5.80	10.52	4.88
		N	10581	8635	5691	14528	5007
2	MP 2	% change	0.28	0.54	0.53	0.82	0.99
		t_R	8.45	7.23	5.84	10.56	4.95
		N	10124	8625	5690	14992	4865
3	pH 1	% change	0.92	0.44	0.72	0.51	1.20
		t_R	8.46	7.25	5.81	10.56	4.92
		N	10323	8748	5621	14326	4808
4	pH 2	% change	0.18	0.77	0.29	0.25	0.43
		t_R	8.50	7.26	5.81	10.52	4.86
		N	10113	8551	5627	14571	4825
5	WL 1	% change	0.12	0.52	0.01	0.92	1.51
		t_R	8.53	7.27	5.80	10.54	4.90
		N	10291	8457	5620	14326	4851
6	WL 2	% change	0.76	0.49	0.49	0.11	1.11
		t_R	8.49	7.26	5.88	10.50	4.95
		N	10323	8254	5626	14326	4805
7	CT 1	% change	0.71	0.17	0.73	0.03	0.86
		t_R	8.47	7.25	5.80	10.53	4.91
		N	10332	8682	5561	14745	4881
8	CT 2	% change	0.49	0.05	0.88	0.27	0.57
		t_R	8.55	7.23	5.81	10.57	4.95
		N	10528	8425	5504	14462	4824

LDT = Lasmiditan; Imp = Impurity; t_R (min) = retention time; N = No. of theoretical plates; all results of average of three analyses

The forced degradation studies were performed to identify the probable DPs that can helpful for establishing the degradation pathway and subsequently the molecules intrinsic stability. The stability indicating ability of an analytical method considered as powerful tool for establishing the products shelf life. Hence, the stability indicating ability of the proposed method was assessed and the degradation products (DPs) formed in the study were characterized using LCMS/MS analysis. The % degradation was calculated to be 6.15 % in acidic, 7.84 base, 5.31 % in peroxide, 3.02% in thermal and 6.85 % in UV light degradation study after 24 h of stress exposer. Based on the degradation results, it was observed that there is no significance degradation was noticed in thermal degradation study without any degradation products detected in the chromatogram. The chromatogram observed in acid degradation study resolve two DPs at the retention time of 1.5 min (DP 1) and 3.1 min (DP 2) along with impurity 2. In the base degradation study, the chromatogram shows well resolved degradation product (DP 3) at the retention time of 3.5 min along with impurity 2 and 4. DP 4 was retained at 6.4 min in the chromatogram of peroxide degradation and the chromatogram also shows the peak corresponds to impurity 4. The chromatogram of UV light degradation study show peak corresponds to DP 5 at the retention time of 12.1 min and the chromatogram also shows the peaks corresponds to DP 4, impurity 2 and 4. The

The purity test results of lasmiditan peak in stress degradation studies suggest that the peak was pure and homogeneous in all studies. The mass balance of 98.08-99.21% was notices in all the studies stress degradation tests. The results noticed for lasmiditan in stress study was insignificant and the peak purity results achieved in stress studies confirms the stability-indicating ability of the proposed method. Table 3 presents the results and Figure 4 shows the representative chromatograms observed in forced degradation study.

Table 3. Forced degradation results of lasmiditan in the proposed method

Stress condition	% degradation[$] of lasmiditan	% assay[$] of lasmiditan	% Mass balance[$] (assay + total impurities	Remark
Acid	6.15	93.85	99.21	DP 1 and 2 were identified
Base	7.84	92.16	98.46	DP 3 and 4 were identified
Peroxide	5.31	94.69	98.53	DP 4 was identified
Thermal	3.02	96.98	98.08	No degradation products separated
UV light	6.85	93.15	98.73	DP 4 and 5 were identified

[$] average of three replicate experiments

3.1. LC-MS/MS Characterization of DPs

The DPs generated in forced degradation of lasmiditan were characterized using LCMS/MS analysis. Based on the retention time of DPs observed in the forced degradation chromatogram, it was confirmed that 5 DPs were formed and were designated as DP 1 to 5. All the DPs along with standard lasmiditan exhibited abundant protonated molecular ions ($[M+H]^+$) in positive ionization mode. The structural information of DPs of lasmiditan was confirmed by recording collision induced dissociation spectra the compounds. The mass fragmentation spectra of DPs formed in the study was presented in the supported file.

The ESI/MS spectrum of DP 1 identified at t_R of 1.5 min show abundant parent ion at *m/z* of 364 $[M+1]^+$ which was formed by losing methyl group ($–C_1H_2$) attached to N-methylpiperidine ring of lasmiditan. The fragmentation spectra shows abundant fragment ion at *m/z* of 163 $[M+1]^+$ due to 2,4,6-Trifluorophenyl)methanol moiety of lasmiditan with molecular formula of $C_7H_5F_3O$ and its proposed structure was presented in Figure 5.

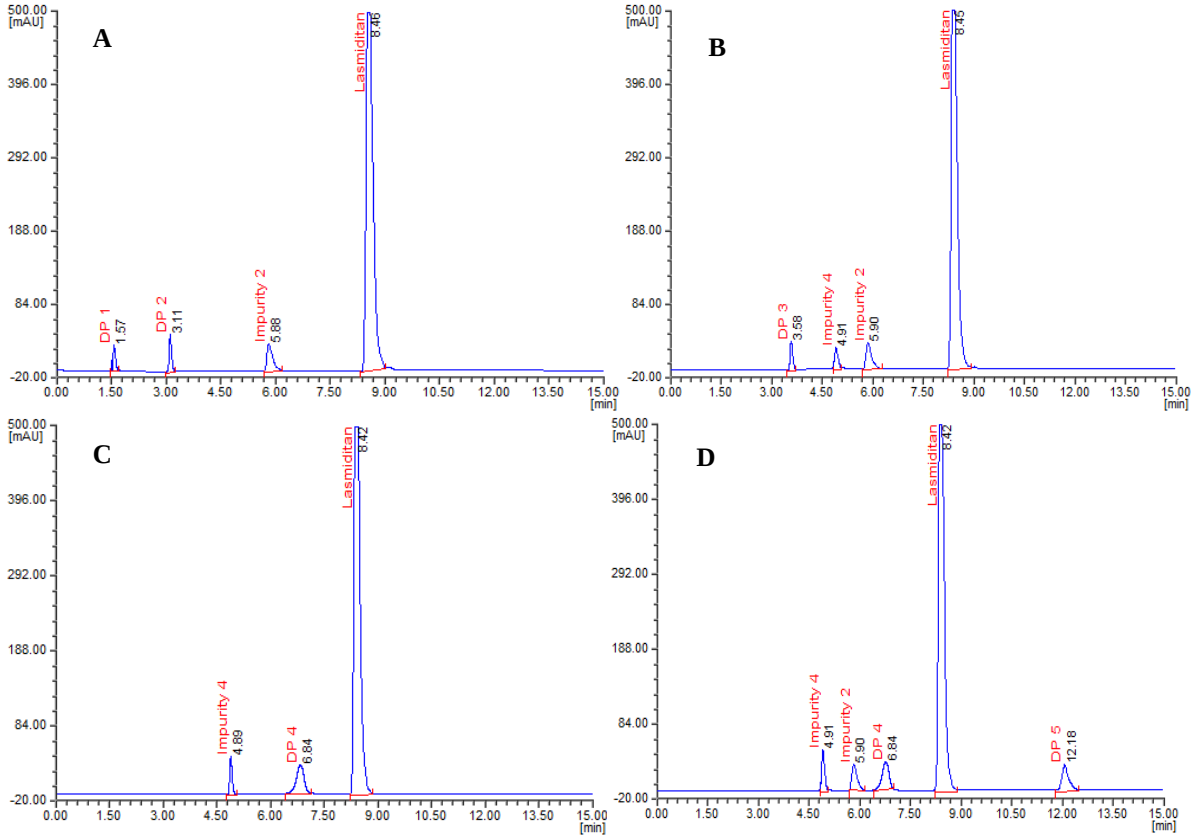


Figure 4. Forced degradation chromatograms of lasmiditan in the proposed method

A) Acid degradation chromatogram showing DP 1 and 2 of lasmiditan; **B)** Base degradation chromatogram showing DP 3 of lasmiditan; **C)** Peroxide degradation chromatogram showing DP 1 of lasmiditan; **D)** UV light degradation chromatogram showing DP 4 and 5 of lasmiditan

In the acid degradation chromatogram, DP 2 was identified at t_R of 3.1 min and was characterized using LCMS/MS study. The fragmentation spectra of DP 2 show fragment corresponds to parent ion at *m/z* of 351 $[M+1]^+$ confirms the molecular weight of DP 2 as 350. The DP 2 was formed due to the breakage of C-N bond of pyridine ring and losing - C_1H_1N of lasmiditan. The fragmentation spectra shows abundant fragment ion at *m/z* of 163 $[M+1]^+$ due to 2,4,6-Trifluorophenyl)methanol moiety of lasmiditan with molecular formula of $C_7H_5F_3O$. Based on the mass spectral results, the DP 2 was confirmed as N-[6-(2-ethylbutanoyl)pyridin-2-yl]-2,4,6-trifluorobenzamide and its molecular structure was presented in Figure 5.

The base degradation chromatogram show DP 3 at t_R of 3.5 min and its mass spectra (Figure 5C) shows parent ion at *m/z* of 176 $[M+1]^+$ was due to $C_7H_4F_3NO$. The DP 3 was formed by losing $C_{12}H_{14}N_2O$ from lasmiditan due to breakage of aromatic N-C bond. The mass spectra also show abundant fragment ion at *m/z* of 159 $[M+1]^+$ suggest the molecular structure of DP 3. Based on the results, the DP 3 was identified as 2,4,6-Trifluorbenzamide (Figure 5).

The peroxide degradation chromatogram retained the DP 4 at t_R of 6.4 min and the product was generated by losing $C_8H_6F_3NO$ of lasmiditan and the compound was confirmed as 2,3-dihydropyridin-4-yl(pyridin-2-yl)methanol with molecular formula of $C_{11}H_{12}N_2O$ and its structure was presented in Figure 5. The mass spectrum shows abundant fragment ion at *m/z* of 94 $[M+1]^+$ which was generated due to lose of C_5H_5NO from DP 4. The same compound was identified in the degradation chromatogram of UV light.

The DP 5 was formed due to UV light stress study chromatogram of lasmiditan. In the mass spectrum of DP 5, characteristic parent ion noticed at *m/z* of 321 with molecular formula of $C_{17}H_{15}F_3N_2O$ which was originated by losing C_2H_3NO. The spectrum also show abundant parent ion at

m/z of 95 $[M+1]^+$ due to $C_5H_6N_2$. The compound was confirmed as 2,4,6-trifluoro-N-[6-(2-methylidenebutyl)pyridin-2-yl]benzamide and its structure was presented in Figure 5.

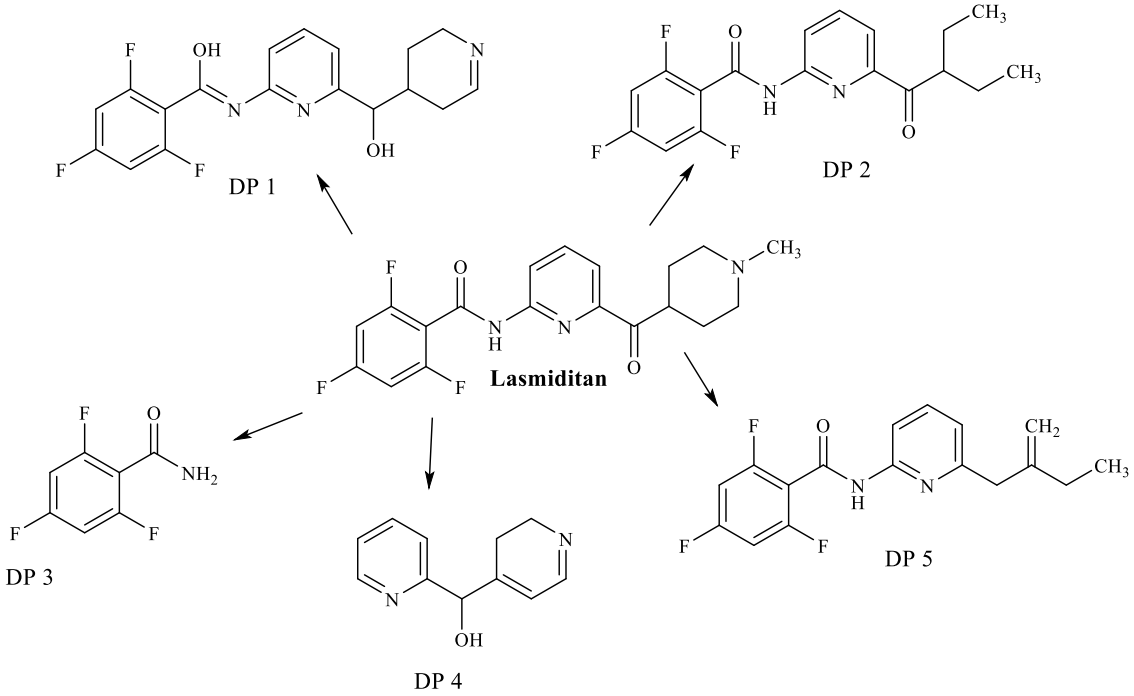


Figure 5. DPs of lasmiditan identified during forced degradation study

The method was adopted for detection and quantification of process related impurities in formulations and in this study, the Reyvow® brand formulation of lasmiditan was selected. The prepared impurities spiked and un-spiked formulation solution of lasmiditan was analysed in the proposed method. The impurities spiked formulation solution clearly resolves the impurities along with standard lasmiditan (Figure 6A). The chromatogram observed for un-spiked formulation solution show peak corresponds to impurity 2 only and other impurities were not detected (Figure 6B). This proved that the impurity 1, 3 and 4 were not present in the formulation or they are less than the detection limit of the method. The % assay of the impurity 2 detected was calculated to be 0.03% which was considered to be negligible. There is no detection of formulation other un-known impurities and formulation excepients in both spiked and un-spiked formulation analysis and hence this method was confirmed to be suitable for the routine analysis of lasmiditan and its process related impurities.

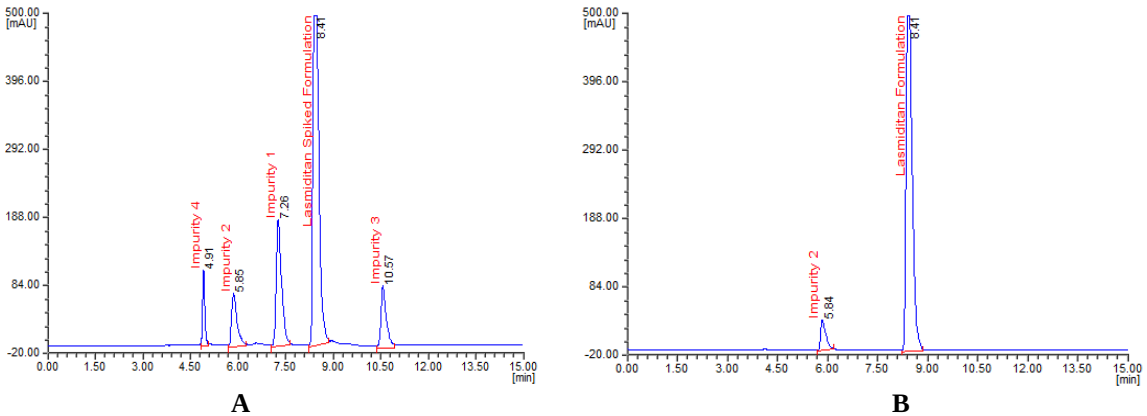


Figure 6. Chromatogram observed during the analysis of Reyvow® formulation solution in the proposed method

A) Reyvow® formulation solution spiked with 0.1 % of each impurity in the study; **B)** Reyvow® formulation solution spiked with no impurities

3.2 Assessment of Uncertainty

The uncertainty calculation in the developed method was performed for the results observed during the analysis of standard solution containing 100 µg/mL of lasmiditan spiked with 0.1 % impurities in the study. The results were expressed in percentage (%) at 95 % confidence level for all the analytes in the calculated parameters. The results noticed in the study were presented in Table 4 which suggests that the results were acceptable. The result related to the uncertainty due to sample weighing was noticed to be negligible and hence were presented.

Table 4. combined and expanded uncertainty results

Analytes	Uncertainty (U) in %					
	$U_{standard}$	$U_{Calibration}$	$U_{Recovery}$	$U_{Repeatability}$	$U_{Combined}$	$U_{Expanded}$
Lasmiditan	0.456	0.284	0.261	0.382	0.709	1.390
Impurity 1	0.716	0.353	0.214	0.351	0.898	1.759
Impurity 2	0.612	0.249	0.422	0.229	0.817	1.601
Impurity 3	0.167	0.331	0.172	0.305	0.511	1.001
Impurity 4	0.191	0.362	0.136	0.149	0.456	0.894

The findings reported in the study were compared with the literature available for the analysis of lasmiditan. The method reported by David Raju, 2012 [7], Santosh Kumar *et al*., 2021 [8] and Harshali *et al*., 2022 [9] were suitable for the analysis of lasmiditan in formulations. These reported method not able to resolve and quantify process related impurities of lasmiditan. Hence present study significantly helpful for quantification of process related impurities and also applicable for characterizing the DPs of lasmiditan.

4. Conclusion

This paper presents a stability-indicating HPLC isocratic method for quantification of process related impurities of lasmiditan. The lasmiditan pure drug was subjected to various stress (acidic, basic, peroxide, thermal and UV light) conditions. The chromatographic response of the stress samples suggests a number of degradation compounds of lasmiditan. The drug was notices to be strongly degraded in acid, UV light condition showing high number of DPs and less degraded under thermal stress with the appearance of no DPs. The structures of the DPs are established by LC-MS/MS and this will be helpful for assessing the degradation pathway and stability of lasmiditan. The isocratic selected HPLC method is validated and is specific, linear, precise and accurate that can helpful for identification and quantification of process related impurities and degradation compounds of lasmiditan.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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References

[1] N. Rahman, S.N.H. Azmi and H.F. Wu (2006). The importance of impurity analysis in pharmaceutical products: an integrated approach, *Accred. Qual, Assur.* **11**, 69-74.

[2] S. Gorog (2006). The importance and the challenges of impurity profiling in modern pharmaceutical analysis, TrAC *TrAC, Trends. Anal, Chem.* **25**, 755-757.

[3] M. Farkkila, H.C. Diener, G. Geraud, M. Lainez, J. Schoenen, N. Harner, A. Pilgrim and U. Reuter (2012). Efficacy and tolerability of lasmiditan, an oral 5-HT1F receptor agonist, for the acute treatment of migraine: a phase 2 randomised, placebo-controlled, parallel-group, dose-ranging study, *Lancet. Neurol.***11**, 405-413.

[4] K.K. Blakely and K. Stallworth (2020). Lasmiditan is a new option for acute migraine treatment, *Nurs. Womens, Health.* **24**, 294-299.

[5] Bernice Kuca, D. Stephen, Silberstein, Linda Wietecha, H. Paul. Berg, Gregory Dozier and R.B. Lipton (2018). Lasmiditan is an effective acute treatment for migraine A phase 3 randomized study, *Neurology*. **91**, 2222-2232

[6] J.H. Krege, P.B. Rizzoli, E. Liffick (2019). Safety findings from phase 3 lasmiditan studies for acute treatment of migraine: Results from SAMURAI and SPARTAN, *Cephalalgia* **39**, 957-966.

[7] M. David Raju (2012), Development and validation of HPLC method for the determination of lasmiditan drug in bulk and tablet dosage form, *J. Pharm, Sci. Res.* **13**, 170-173.

[8] L. Santosh Kumar, Valmiki and P. Yadagiri Swamy (2021). RP-HPLC method development and validation of lasmiditan in bulk drug and pharmaceutical dosage form, *J. Emerg. Technol. Innov. Res.* **8**, 54-61.

[9] S. Harshali, A. Ritesh, K. Sarita and R.L. Kavita (2022). HPLC method development and validation for the estimation of lasmiditan in marketed formulation, *World. J Pharm. Res.* **11**, 2022-2032

[10] D. Dinesh, B.S. Bhaskar, P. Chand, S. Naik, S. Badgujar and D. Baviskar (2020). Process of preparation of lasmiditan, world intellectual property organization, publication No: WO 2020/095171 A1.

[11] B.K. Bikshal, R.M. Useni, R.A. Venkateswara and R.L. Maheshwara (2018). Intended high-performance liquid chromatography procedure for the quantification of norfloxacin and its potential impurities in active pharmaceutical ingredient and tablet dosage forms. *Thai. J. Pharm. Sci.* **42**, 27-36.

[12] U.R. Mallu, V.R. Anna and B.B. Kasimala (2019). Rapid stability indicating HPLC method for the analysis of leflunomide and its related impurities in bulk drug and formulations, *Turk. J. Pharm. Sci.* **16**, 457-465.

[13] B. B. Kasimala, V.R. Anna and U.R. Mallu (2018). Stability-Indicating reversed-phase HPLC method for the separation and estimation of related impurities of cilnidipine in pharmaceutical formulations, *Indian Drugs.* **55**, 41-49.

[14] K. S. Girija, B.B. Kasimala and V.R. Anna (2021). A new high-performance liquid chromatography method for the separation and simultaneous quantification of eptifibatide and its impurities in pharmaceutical injection formulation, *Int. J. App. Pharm*. **13,** 165-172.

[15] ICH validation of analytical procedures: text and methodology Q2(R1). 1994. 1-13.

[16] A. Kul (2022). Simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol by HPLC-PDA in pharmaceutical dosage forms, *J. Chem. Metrol*. **16,** 102-110

[17] B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics, (2nd ed. 2014). ISBN 978-91-87461-59-0