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LC/MS/MS Method Development and Validation for the Estimation of Lasmiditan in Bulk and Pharmaceutical Formulation

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ABSTRACT

Background: Lasmiditan is an agonist of 5HT1F receptor, acute migraine is treated and oral dose is safe and effective. No analytical methods were reported on Lasmiditan Drug. Objectives: The goal of the current project is to create a quick, effective, and reproducible LC/MS/MS method for the estimation of Lasmiditan. The mobile phase was used Acetonitrile: 0.1% formic acid (70:30) in the developed method, and it was running down a C18 column (SP) with dimensions of 150 mm, 4.6 mm i.d., and 3.5 µm at a rate of 1 mL/minute. The $[M+H]^+$ ion m/z ratio for the substance was observed in the mass spectrum was 378.24. The drug had a retention time of 2.3 minutes. The linearity range was found to be in between 12.50 ng/mL and 75 ng/mL. The regression coefficient value was found to be 0.999, which is good satisfactory value for linearity. The LOD and LOQ for baseline measurement and detection were 0.66 ng/mL and 2.22 ng/mL, respectively; the method was shown good accuracy and precision levels, and it was proved through the assay values. The method was proved as robust after deliberated changes in parameters of flow rate (±0.2mL) and organic phase (±3 mL) in mobile phase composition and values were found to be 99.50%-100.73% & 99.10%- 101.83% respectively. Conclusion: The LC/MS/MS method is more advanced and sensitive to determine the drugs at nanograms level. Hence developed method used for the regular assay. This is more sensitive, selective, and rapid for identification and quantification of LMT in the bulk and Pharmaceutical formulation.

Keywords: Lasmiditan, LC/MS/MS, Acetonitrile, Formic acid.

INTRODUCTION

The Lasmiditan (Fig. 1) drug is used for the treatment of acute migraine and available in tablet dosage form. It lacks vasoconstrictive properties, safe and effective for the treatment of acute migraine in the patients with cardiovascular risk factors in which triptans are contraindicated.[1-3] Lasmiditan is

a selective 5 HT_{1F} receptor agonist and less affinity on 5 HT_{1B} receptor does not cause vasoconstriction.[4] For the treatment of an acute therapy for migraine Lasmiditan being developed to address significant unmet needs with risk factors with the cardiovascular system, poor response to the current treatment and stable cardiovascular disease.[5-12] The oral dose of Lasmiditan is safe and more effective in the treatment of acute migraine.[13,14] The RP-HPLC[15-17], impurities in Lasmiditan[18] and Bio-analytical[19] methods were on reported LMT. The developed method was more sensitive and determined at lowest level compared with other reported methods.

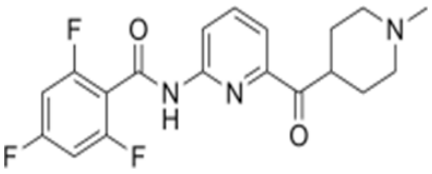


Fig. 1. Lasmiditan molecular structure

MATERIALS AND METHOD

Materials

The Lasmiditan drug was gifted by Supriya Lifescience Ltd. Acetonitrile, formic acid and water for HPLC grade brought from the Merck chemicals. The supplier of these chemicals is Bros Scientifics, Tirupathi, Andhra Pradesh 517507. The HPLC system was coupled to SCIEX QTRAP 5500 (Manufactured by AgilentLC) and ZORBAXLC column was used, it was symmetry C18 150mm×4.6mm i.d and 3.5 µ. The filters were used in the pore size of 0.44 µm, manufactured by Millipore.

Preparation of standard solution

"Lasmiditan was weighed 10 mg, dissolved with diluent and volume was made up to mark (10 mL) with diluent (1000 µg/mL). This is used as a primary standard stock solution. The further dilution was made by transferred 4.5 mL of Apalutamide into 100 mL volumetric flask and made the volume up to mark with diluent. The final concentration of the solution was obtained 45 µg/mL and used as a standard working solution."

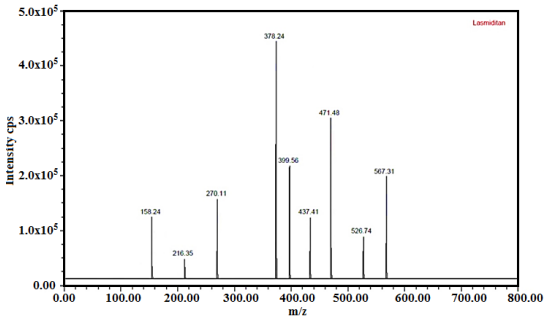
Preparation of marketed formulation (Assay Procedure)

Accurately weighed 20 tablets and calculated average weight of the tablets. The tablets were crushed into powder with the help of mortar and pestle, the powder equivalent to 114.14 mg of Apalutamide was transferred into 10 mL volumetric flask added few quantities of diluent and dissolved by sonicated for 5 min, volume was made up to mark with diluent (Primary stock sample solution). The solution was filtered through 0.22 µL. Further dilution was made with 4.5 mL of solution to 100 mL with diluent and concentration of the solution was obtained 45 µg/mL and it was used as a working sample solution.

RESULTS AND DISCUSSION

Mass Spectrometry condition

The mass spectrometer was operated with mode of positive ion with MRM. Multiple reaction monitoring (MRM) was applied to quantify the Lasmiditan. The HPLC system was coupled to SCIEX QTRAP 5500 and set up the different optimized conditions for the analysis of the selected molecule: collision energy 15V, Ion spray voltage 5500, source of temperature 550, 120-250 temperature of drying gas, nitrogen was used as collision gas, flow stream of drying gas maintained at 5L/min, entrance potential was 10V and declustering potential was 40V. The monitored $[M+H]^+$ ion m/z ratio was 378.24 (Fig. 2). The exit potential was 7V and dwell time was set to 1 second.



Multiple Reaction Monitoring-MRM of the Lasmiditan using Positive Polarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Lasmiditan	378.24	471.48

Fig. 2. Mass Spectrum of Lasmiditan

Method Development

The method was optimized with different conditions and shown in the Table 1.

Analytical Method Validation

The optimized method was validated

according to ICH Q2R1 guidelines and discussed different validation parameters.[20]

Table 1: Optimized conditions for estimation of Lasmiditan by LC/MS/MS method

S. No	Parameter	Description
1	Stationary Phase	Symmetry C18 (150X4.6X5)
2	Mobile Phase	ACN:0.1% Formic acid (70:30)
3	Rate of flow	1 mL/min
4	m/z ratio	378.24
5	Detector	Tandem Quadrupole (Triple Quadrupole) MS
6	Injection	Auto sampler
7	Volume of injection	10 µL
8	Temperature of column	Ambient
9	Run time	4mins
10	Diluent	Mobile Phase
11	Rt	Lasmiditan: 2.33

System suitability

To evaluated tailing factor, resolution and theoretical plates in the system suitability test. The %RSD was calculated for the 100% of standard solution. The chromatograms were recorded for six replicated injections.

Specificity

The standard and sample chromatograms (Fig. 3 & 4) were shown specificity of the method and record the chromatograms at100% concentration of sample & standard solution. The optimized conditions were shown in Table 2.

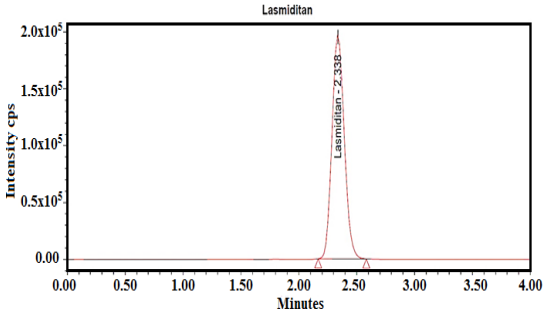


Fig. 3. Chromatogram of Lasmiditan for Standard drug

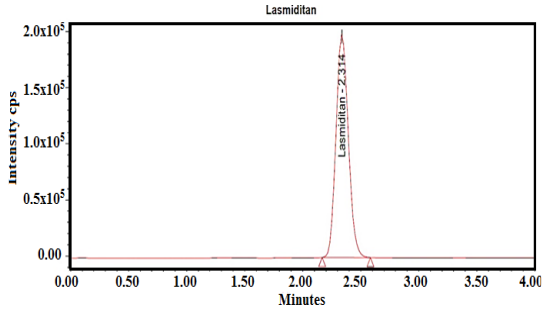


Fig. 4. Chromatogram of Lasmiditan for Marketed formulation

Table 2: System suitability parameters of LMT

S. No	Parameters	LMT	
		Results	Limits
1	Plate Count	15890	>2000
2	USP Tailing factor	1.02	T2
3	USP Resolution	-	R2
4	Retention time	2.33 min	-

Linearity

The calibration curve of the LMT was linear over concentration range, a graph was plotted response of the area on X-axis and concentration on Y-axis and (R^2) regression coefficient value, was found 0.999. The concentration of standard solution shown linearity range from 12.50 ng/mL to 75 ng/mL and graph shown in Figure 4.

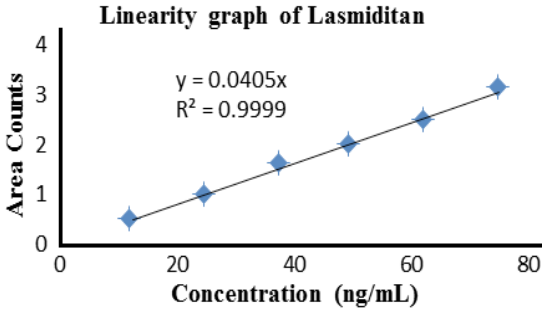


Fig. 4. Linearity graph of Lasmiditan

Precision

The precision was studied at intraday and intermediate precision and every level of precision was evaluated (Table 3) with six replicated injections of Lasmiditan.

Intraday Precision

The intraday precision was performed with 100% sample solution at three time intervals like 9:00 am, 1:00 pm and at 5:00 pm. Record the chromatograms at these three intervals at each level six replicated injections were injected and calculate %RSD.

Interday Precision

The interday precision was performed at day 1, day 2 and day 3 with 100% concentration of solution. Record the chromatograms of six replicated injections at each level and calculated average of %RSD for three days.

The following formula used for the calculation of percentage assay

%Assay=Area in sample/Area in STD× Weight of STD/Dilution of STD×Dilution of sample/Weight of sample×Average weight/Labelled claim× Purity of ST

Table 3: Precision results of Intraday and Interday of LMT

S. No	Lasmiditan			
	Intraday		Interday	
	Peak Area	% Assay	Peak Area	% Assay
1	2.014	98.8	2.012	98.9
2	2.019	99.5	2.020	99.1
3	2.021	99.5	2.011	98.6
4	2.013	99	2.009	99.0
5	2.035	100.3	2.023	99.5
6	2.045	101	2.035	100.4
Mean	2.024	99.7	2.018	99.3
SD	0.01	0.828	0.01	0.635
%RSD	0.49	0.83	0.49	0.64

*Average of three interval

Accuracy

The accuracy at three distinct concentration levels of 50%, 100%, and 150% was evaluated using the recovery studies (Table 4). Six repeated injections at 50% and 150% concentrations and three replicated injections at 100% concentration were used to obtain the accuracy-based recovery percentage.

Table 4: Accuracy results of Lasmiditan

Parameters	Peak Area	Amount added(ng)	Amount recovered (ng) Lasmiditan	% of Recovery	%Mean recovery
**50%	1.027	2.523	2.536	100.53	100.53
*100%	2.045	5.049	5.054	100.10	100.10
**150%	3.057	7.513	7.550	100.52	99.52

Detection limit and quantification limit

The detection limit and quantification limit were determined through the linearity curve of slope and the response of the standard deviation (precision). The LOD and LOQ for LMT were determined to be 0.66 ng/mL and 2.22 ng/mL, respectively.

Robustness

The robustness was performed (Table 5) with only minor adjustments to the method's flow rate and composition of mobile phase, both of which were assessed at 100% sample concentration. The rate of flow was changed±0.2 mL/min and recorded the chromatograms for six replicate injections. The mobile phase composition of the method was changed to ±7mL of organic phase and recorded the chromatograms for six replicated injections.

Table 5: Robustness results of Lasmiditan

Parameter	Condition	LMT		
		Rt (min)	Peak Area	% Assay
Flow rate	0.8 mL/min	2.91	2.323	99.82
	1 mL/min	2.33	2.022	99.50
	1.2 mL/min	1.95	1.865	100.73
Mobile Phase	M:B 63:37v/v	2.52	2.155	100.22
	M:B 70:30v/v	2.33	2.021	99.10
	M:B 77:23v/v	2.20	1.938	101.83

CONCLUSION

The Lasmiditan was estimated using LC/MS/MS in both its bulk and commercial formulation. The technique was successfully validated by following the ICH Q2R1 recommendations. Multiple reaction monitoring was applied to determine the Lasmiditan. The HPLC system was coupled to SCIEX QTRAP 5500 and set up the different optimized conditions for the analysis of the selected molecule. The monitored $[M+H]^+$ ion m/z ratio was 378.24. The method was shown a good linearity range and values found to be 12.50 ng/mL to 75 ng/mL. The regression coefficient value was found to be within the limits, 0.999. The %RSD for accuracy and precision was found to be <2%, it indicated that the parameters are within the limits as per guidelines. The method was proved as robust after deliberated changes in parameters of rate of flow (±0.2 mL) and organic phase (±0.7 mL) in the composition of mobile phase and values were found to be 99.50%-100.73% & 99.10%-101.83% respectively. The LOD and LOQ of the study proved the sensitivity of the method and determine the drug at nanogram level and values were found to be 0.66 ng/mL and 2.22 ng/mL, respectively. The developed method was more sensitive and selective, hence the recommended method allows industrialists and researchers to identify and estimate Lasmiditan in bulk and in commercial formulations.

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Conflict of interest

The authors have declared that they have no competing interests.

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