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HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LASMIDITAN IN MARKETED FORMULATION

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ABSTRACT

A reliable, accurate and simple RP-HPLC method was developed for the simultaneous estimation of Lasmiditan, validated according to ICH guidelines in tablet dosage form. A column of inertsil ODS (150x4.6mm, 3.5µm) with a flow rate of 1ml/min was used. The combination of 0.1% ortho phosphoric acid and Acetonitrile in 50:50 ratio was used as a mobile phase. Lasmiditan peak was eluted at a retention time of 3.203 min. The total run time was 5min. Standard solutions were prepared by dissolving in acetonitrile first and then make up to the mark with mobile phase. The method shows a good linearity in the concentration range of 5-75µg/ml of Lasmiditan with correlation coefficient 0.999. This method was validated in terms of specificity, linearity, accuracy, LOD, LOQ, robustness and forced degradation.

KEYWORDS: RP-HPLC, Development, Validation, Lasmiditan.

INTRODUCTION

Lasmiditan, sold under the brand name Reyvow, is a medication[1,2] used for the acute (active but short-term) treatment of migraine[3,4] with or without aura (a sensory phenomenon or visual disturbance) in adults. It is not useful for prevention. It is taken by mouth. Common side effects include sleepiness[5,6], dizziness[7], tiredness[8], and numbness. There is a risk of driving impairment while taking lasmiditan. People are advised not to drive or operate machinery for at least eight hours after taking lasmiditan, even if they feel well enough to do so. People who cannot follow this advice are advised not to take lasmiditan. The drug causes central nervous system (CNS) depression[9,10], including dizziness and sedation.[11] It should be used with caution if taken in combination with alcohol or other CNS depressants.[12]

Drug profile

Mol. Weight: 377.367

Chemical Formula: C19H18F3N3O2

IUPAC Name: 2, 4, 6-trifluoro-N-[6-(1-methylpiperidine-4-carbonyl) pyridin-2-yl] benzamide.

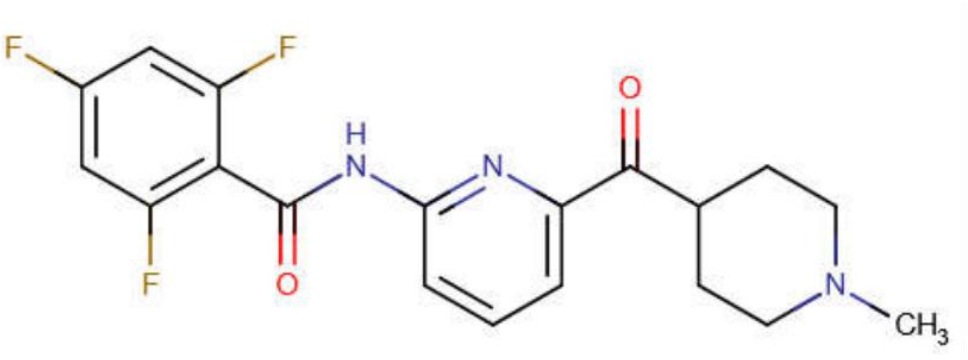

Fig. No. 1: Structure of Lasmiditan.

Pharmacology

Indication: Lasmiditan is indicated for the acute treatment of migraine with or without aura in adults.

Pharmacodynamics: Lasmiditan belongs to a new and novel class of acute anti-migraine medications that exert their effects via inhibition of neuronal firing rather than vasoconstriction of cerebral arteries. Lasmiditan appears to have a relatively quick onset of action (an important characteristic in acute migraine treatment) with some patients reporting benefit within 20 minutes. Due to its ability to cause CNS depression (e.g. drowsiness, dizziness), lasmiditan may cause significant driving impairment and patients should be advised not to participate in activities requiring mental alertness for at least 8 hours after dosing.

Mechanism of action: The acute treatment of migraine headaches has, in the past, been achieved via constriction of cerebral blood vessels, as the acute dilation of these vessels observed during migraines was thought to be the cause of the associated pain. The neurogenic hypothesis of migraine pathophysiology, an alternative to the vascular hypothesis, suggests that cerebral vasodilation is a secondary mechanism in migraine pathogenesis, and that the main contributor to migraine headache pain is the increased pathogenic firing of trigeminal nerve pathways. While the precise mechanism of action of lasmiditan is unclear, it likely

supports this neurogenic hypothesis by exerting its therapeutic effects through potent and selective agonism of the 5-HT1F receptor. 5-HT1F receptors are found in both the central and peripheral nervous system (on the central and peripheral ends of trigeminal neurons) and appear to contribute to hyperpolarization of nerve terminals and inhibition of trigeminal neuronal activity. Lasmiditan's agonism at these receptors may, therefore, inhibit the firing of trigeminal nerves responsible for migraine headache pain.

Absorption: Oral absorption of lasmiditan is quick, with a median tmax of 1.8 hours.

Metabolism: The hepatic and extra-hepatic metabolism of lasmiditan is catalyzed primarily by non-CYP enzymes, with ketone reduction appearing to be the primary pathway.

Route of elimination: Lasmiditan is eliminated primarily via metabolism, with renal excretion accounting for a small fraction of its total elimination. Of the small amount of drug found in the urine post-dose, approximately 66% is comprised of lasmiditan's S-M8 metabolite. Only 3% of an administered dose of lasmiditan was recovered unchanged in the urine, further implying a relatively extensive metabolism of this drug.

Half-life: The mean elimination half-life of lasmiditan is 5.7 hours.

Uses: Lasmiditan is used to treat migraines. It helps to relieve headache, pain, and other migraine symptoms (including nausea, vomiting, and sensitivity to light/sound).

Side effects: Drowsiness, dizziness, tiredness, or numbness/tingling of the skin or mouth.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile, water and methanol were purchased from Merck specialties pvt, Ltd., Mumbai, India. API of Lasmiditan as reference standards were procured from Bio plus life science, Bangalore. Label claim of LSD in tablet is 50mg respectively. Reverse Osmosis Water was used throughout the study.

Instrumentation

FT IR spectrophotometer (Bucker alpha/opus 7.8), Analytical Balance (AUX-200), UV Visible.

Spectrophotometer (Labindia 3000 Plus), HPLC(Shimadzu), Ultrasonic Water Bath were used.

Preparation of stock and working standards Preparation of standard solution: Accurately weighed 10mg API of LSD was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000□g/ml (Stock-A)

Preparation of Sub Stock Solution

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of LSD. (Stock-B).

Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for LSD.

Method Validation

The analytical method was validated as per ICH Q2 (R_1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, LOD, LOQ, forced degradation and stability.

System suitability

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of LSD 1□g/ml were injected. Peak report and column performance report were recorded for all chromatogram.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Precision

The stock solution was prepared. The precision are established in three differences:

1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5□g/ml for LSD indicates the precision under the same operating condition over short interval time.

2. Intermediate Precision

A) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in three replicate at five concentrations.

B. Analyst to Analyst

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 10mM KH2PO4: Methanol (30:70 % v/v) to (35:65 % v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Linearity and range

Linearity was conducted by preparing different standard solutions of Lasmiditan at different concentration levels. The standard solutions were prepared in the concentration range of 5-75µg/ml of Lasmiditan. Each concentration was injected into the HPLC system and record the areas obtained. Plot a graph between area taken on Y-axis and concentration on X-axis.

LOD and LOQ

LOD was measured by diluting the standard solution of Lasmiditan and determining the concentration was response of sample peaks are three times the noise peak. LOQ was

measured by diluting the standard solution of Lasmiditan and determining the concentration was response of sample peaks are ten times the noise peak.

RESULTS AND DISCUSSION

Method development and optimization

The most suitable isocratic condition to resolve Lasmiditan with inertsil ODS column, after the chromatographic conditions were optimized for specificity, resolution and retention time was a mobile phase consisting of 0.1% OPA and Acetonitrile in the ratio of 50:50. When a higher percentage of mobile phases was used, the resultant chromatogram had an increase either in back ground noise or peaks indicating the tailing effect. Thus based on the above mentioned parameters, Lasmiditan were eluted at a retention time of 3.203 min.

Table 1: HPLC isocratic method for Lasmiditan.

S. No.	Parameter	Method Conditions
1	Column	Inertsil ODS 150x4.6mm, 3.5µ
2	Flow rate	1 ml/min
3	Wave length	258nm
4	Injection Volume	10µl
5	Run time	5 min
6	Mobile phase	0.1% OPA: ACN 50:50

Method Validation

The method was validated according to the validation of analytical procedures provided in the ICH guidelines and draft guidance for the industry, analytical procedures and method validation.

System suitability

The standard solution was introduced into the HPLC system and found that system suitability parameters are within the limits. The %RSD was calculated to standard peak areas. The system precision results were tabulated in table 2 and the chromatogram of standard was exhibited in the Table No 2.

Table No 2: Results of system suitability parameters.

Parameters	% MEAN±SD*
	LSD
No. of Theoretical Plates	2561.500±10.075
Tailing Factor	1.255±0.036
Retention time	3.448±0.022

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Linearity

Lasmiditan linearity concentration was prepared in the range of 5-75µg/ml. The regression equation was found to be Y= 24109.03x+47536.14 and correlation coefficient was 0.9997. Result showed in table no 3.

Table 3: Results of linearity.

PARAMETER	LSD
Concentration (µg/ml)	1-5
Correlation Coefficient	**(r2)*** 0.999
Slope (m)*	32.31
Intercept (c)*	5.141

Robustness

In robustness there is a small deviation in flow rate (±0.2ml) and organic solvent (±10%) in their chromatographic condition and observed that there is no significant change in %RSD. Results of robustness showed in table no 4.

Table 4: Results of robustness.

Parameter	% MEAN±SD*
	LSD
Robustness	**97.595±0.041**

***** Value of five replicate and five concentrations.

Stability

Stability of Lasmiditan was determined in sample solution was studying initial to 24hr at different time intervals at room temperature. The results indicate that there is no significant deviation of purity. Results of stability showed in table no 5.

Table 5: Results of stability.

S.No	Stability	Purity of Lasmiditan in RT	Purity of Lasamiditan in 2-8°C
1	**Initial**	**99.9**	**99.9**
2	**6Hr**	**99.6**	**99.5**
3	**12Hr**	**99.2**	**99.1**

4	**18Hr**	**99.0**	**98.9**
5	**24Hr**	**98.7**	**98.6**

Precision

Precision of the method was established by injecting test preparation and tested through the complete analytical procedure from sample preparation to the final result. Result of method precision showed in table no 6.

Table 6: Results of system precision.

Parameter	**% MEAN±SD***
	LSD
Repeatability	**97.831±0.046**
Intermediate precision	
Day to day precision	**97.854±0.042**
Analyst to analyst	**98.558±0.040**

***** Value of five replicate and five concentrations.

Limit of Detection and Limit of Quantification (LOD & LOQ)

The LOD concentrations of Lasmiditan were 0.063µg/ml and LOQ concentrations of Lasmiditan was 0.206µg/ml. Result of LOD and LOQ showed in table no 7.

Table 7: Result of LOD and LOQ.

Name	**LOD (µg/ml)**	**LOQ (µg/ml)**
LSD	0.15	0.45

Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentration levels (50%, 100% and 150%). APIs with concentration 25, 50 and 75µg/ml of Lasmiditan were prepared. The percentage recovery values were found to be in the range of 98-102%. **Results of Accuracy of Lasmiditan showed in table no 8.**

Table no 8: Results of Accuracy of Lasmiditan.

% Level	**% MEAN±SD***
	LSD
80%	99.98±0.210
120%	97.78±0.960
80%	98.69±1.040

Assay of tablet

The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drug. **Result of Assay of tablet showed in table no 9.**

Table no 9: Result of Assay of tablet.

	LSD*
Label Claim (mg)	**50mg**
% found	49.78
% Assay	99.56
% RSD	0.092

*Average of three determination.

DISCUSSION

In the present research work, a successful attempt was made for **"Validated UV and HPLC method development for the estimation of Lasmiditan in marketed formulation"** which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation.

Liquid chromatographic system from waters comprising of manual injector, Waters 515 binary pump for constant flow and constant pressure delivery and U.V. detector connected to data ace software controlling the instrumentation as well as processing the data generated were used. The isocratic mobile phase consisted 10mM KH2PO4: Methanol (pH 3.5 with OPA) in the ratio of 30:70v/v in the ratio of 70:30v/v at a flow rate of 1.0 ml min-1. A thermo C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 260.0 nm was selected as the detection wavelength for UV-vis. detector.

CONCLUSION

The proposed methods were found to be **linear in the range of 5-25µg/ml with correlation coefficient close to one.** Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent. The result obtained shows the developed methods to be **Cost effective**, **Rapid** (Short retention time), **Simple**, **Accurate** (the value of SD and %RSD less than 2), P**recise** and can be successfully employed in the routine analysis

of these drugs in bulk drug as well as in tablet dosage form. **The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.**

Conflicts Of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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