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Development and Validation of HPLC Method for the Determination of Lasmiditan Drug in Bulk and Tablet Dosage Form

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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.

Key words: RP-HPLC, Development, Validation, Lasmiditan.

1. 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].

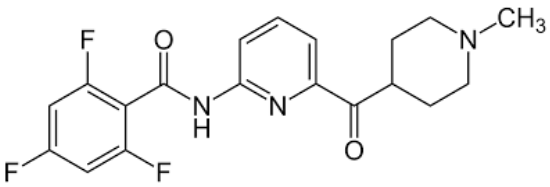

Fig. No. 1: Structure of Lasmiditan

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Acetonitrile, ortho phosphoric acid (OPA), water and methanol were purchased from Merck (India) Ltd. Worli, Mumbai, India. API of Lasmiditan as reference standards were procured from Dr Reddys Laboratories, Hyderabad.

2.2 Instrumentation

Waters alliance e-2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

2.3 Preparation of stock and working standards

Preparation of standard solution: Accurately weighed 50mg of Lasmiditan working standards were transferred into 100ml volumetric flask. Add approximately 70ml of diluents and sonicated for 15min to dissolve the components. After 15min. makeup to the mark with diluents. Further diluted 5ml of the above solution to 50ml volumetric flask and diluted to volume with diluents.

Preparation of Sample solution: 2 tablets were weighed and crush the 2 tablets into powder form, take the sample equivalent to 50mg of Lasmiditan was transferred into a 100ml volumetric flask and add 70ml of diluents and sonicated for 50mins to dissolve the components and then diluted up to the mark with diluents. Further dilute 5ml of the above solution to 50ml with diluents and it was filtered through 0.45µ nylon syringe filter.

2.4 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.

2.4.1 System suitability

System suitability parameters like USP plate count, USP tailing and %RSD were measured and found to be within the limits.

2.4.2 Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was studied at three different concentration levels (50%, 100% and 150% levels). Minimum three injections were given in each level and percentage of recovery, % RSD was calculated.

2.4.3 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components (impurities, degradates or excipients), which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Lasmiditan.

2.4.4 Precision

In this method precision was evaluated as system precision, method precision and intermediate precision.
In system precision six replicate standard solutions of Lasmiditan were analyzed and %RSD was calculated.
In method precision six preparations with sample were injected and %RSD, % recovery were calculated. The intraday and inter-day precision study was conducted for Lasmiditan.

2.5.5 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.

2.4.6 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.

2.4.7 Stress degradation studies

Forced degradation studies were used to evaluate the specificity of the method. The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

2.4.8 Robustness

In robustness the method was determined by making slight changes in the flow rate ±20%, organic phase ±10%, wave length by ±5nm.

24.9 Stability

Analytical solution was prepared and injected into the HPLC system at time intervals between 0 hours to 24 hours at 6hours intervals depending on the instrument utilization and sequence of injection.

3. Results And Discussion

3.1 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 depicts the chromatographic parameters applied for the method.

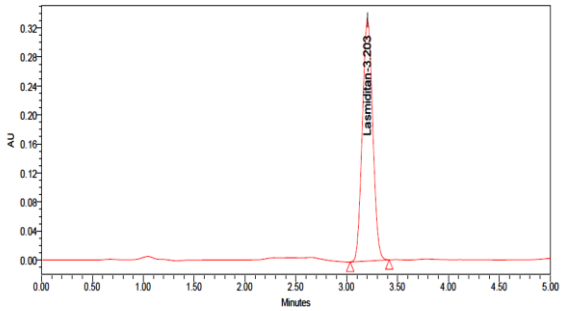

Fig. No. 2: Representative chromatogram of Lasmiditan

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

3.2 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 figure 3.

Table 2: Results of system precision

S. No	System suitability parameter	Acceptance criteria	Lasmiditan
1	% RSD	NMT 2.0	0.62
2	USP Tailing	NMT 2.0	1.02
3	USP Plate count	NLT 3000	3659

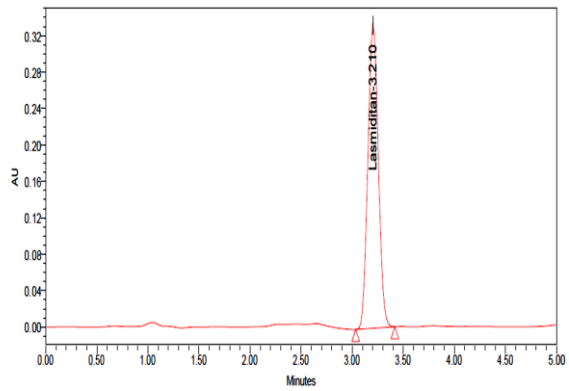

Fig. No. 3: Chromatogram of standard

Specificity

In specificity samples were prepared by adding equivalent weight of API and placebo with test concentration and then injected into HPLC system. Interference was not found for the chromatograms of placebo solution, empty cell solution and impurities at the retention time of Lasmiditan.

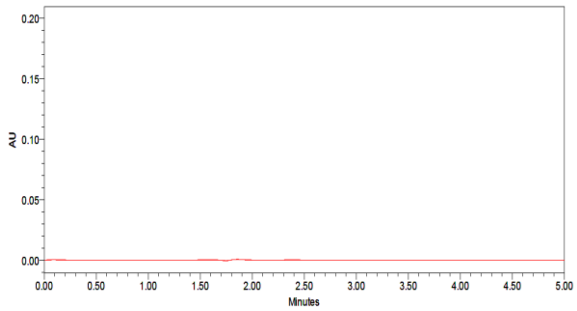

Fig. No. 4: Chromatogram of blank

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.

Table 3: Results of linearity

S. No.	Lasmiditan	
	Conc. (µg/ml)	Area
Linearity-1	5.00	687456
Linearity-2	12.50	1254897
Linearity-3	25.00	1879421
Linearity-4	50.00	2464894
Linearity-5	62.50	3068745
Linearity-6	75.00	3634583
Slope	24109.03	
Intercept	47536.14	
CC	0.9997	

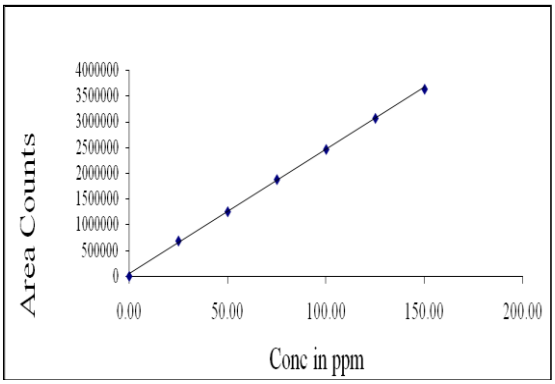

Fig. No. 5: Linearity plot of Lasmiditan

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.

Table 4: Results of robustness

S.No	Parameter name	% RSD of Lasmiditan
1	Flow (0.8ml/min)	0.14
2	Flow (1.2ml/min)	0.29
3	Organic solvent (+10%) (55:45)	0.83
4	Organic solvent (-10%) (45:55)	0.51

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.

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.

Table 6: Results of method precision

Analyte	Std Conc.	%RSD
Lasmiditan	50	0.11

Intermediate Precision

Six replicates of a sample solution was anlaysed on a different day, different analyst and different RSD values.

Table 7: Results of Intermediate precision

Analyte	Std Conc.	%RSD
Lasmiditan	50	0.55

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.

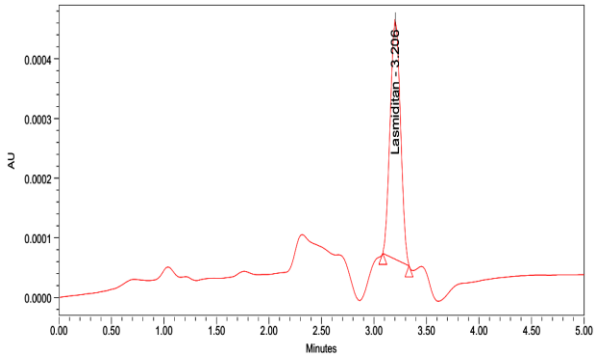

Fig. No. 6: Chromatogram of LOD

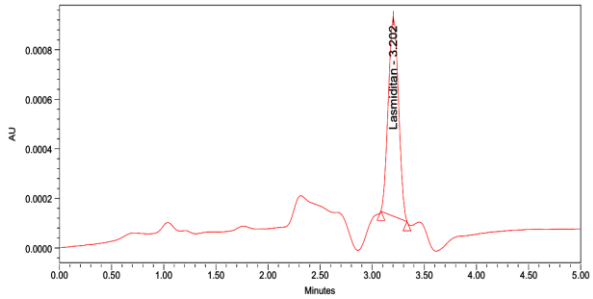

Fig. No. 7: Chromatogram of LOQ

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%.

Table 8: Results of Accuracy of Lasmiditan

S. No.	% Level	% Recovery	Ave % Recovery
1	50	100.4	100.4
2		100.1	
3		100.6	
4	100	99.2	99.3
5		99.0	
6		99.7	
7	150	99.8	99.9
8		98.9	
9		100.9	

Degradation effects

Lasmiditan sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Forced degradation studies were performed to show the method is suitable for degraded products. Moreover, the studies provide information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities.

Forced degradation results were tabulated in table 9.

Table 9: Results of Forced degradation of Lasmiditan

Degradation Condition	% Degradation
Unstressed Degradation	0.1
Acid Degradation	15.8
Alkali Degradation	15.3
Peroxide Degradation	14.5
Reduction Degradation	12.7
Thermal Degradation	13.9
Photolytic Degradation	12.1

4. Discussion

In the present study, to elute Lasmiditan we use reverse phase HPLC, inertsil ODS column, 0.1% OPA and acetonitrile (50:50) as mobile phase. The reliability, accuracy and precision within the ICH and FDA limits for the method validation of analytical samples. In addition, analysis of the marketed preparation of Lasmiditan with the validated assay methods showed that the drug contents eluted with no interfering peaks generated by the excipients in the marketed products. Results for robustness and the method were found to remain unaffected by changing the method parameters.

5. Conclusion

A validated RP-HPLC method for stability indicating assay of Lasmiditan was developed. The degradation behaviour of the drug was investigated under hydrolysis (acid, base and neutral), oxidation, photolysis and thermal stress conditions. The drug was found to best able in basic, neutral conditions and unstable in oxidative conditions.

An isocratic RP-HPLC method for the determination of Lasmiditan was developed and is precise and reliable. The regression line equation is capable of reliably predicting the drug concentration in the range of 5-75 µg/ml of Lasmiditan, from the peak area obtained. The method was successfully validated and allowed the reliable, sensitive, robust and specific detection of Lasmiditan in a marketed preparation.

Conflicts Of Interest

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

Acknowledgement

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