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Spectroscopic Method Development and Validation for Lasmiditan Quantification in Bulk and its Tablet Formulations

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A simpler, economical visible spectroscopic procedure for determining the value of Lasmiditan in bulk active pharmaceutical ingredients and its formulation is developed. It is used in the acute treatment of migraines. Lasmiditan helps reduce the risk of vasoconstriction, making Lasmiditan a suitable option for patients with cardiovascular conditions. The absorption maximum was found to be 626 nm. As per Beer Lambert's law, the linearity was in the 10-50 μg/ml concentration range. Validation parameters such as Molar absorptivity, Accuracy, Precision, and Sandal's sensitivity were within the regulated limits. The Percentage Relative Standard Deviation was less than 10% and the average recovery was 98%. This quantitative analytical method is used in quality control laboratories, bulk drugs, and formulation industries.

Keywords: Bulk Drug; Estimation; Formulation; Lasmiditan; Spectroscopy.

Lasmiditan is a type of drug called a Ditan. In 2020, Lasmiditan was the first Ditan approved by the US-FDA. Lasmiditan works by blocking the pain pathways in the brain. Lasmiditan blocks the serotonin $(5\text{-HT})_1$F receptor, but its exact mechanism of action is unknown[1,2,3]. Unlike Triptans, which are the gold standard of treatment for migraines. Lasmiditan will show its action without causing vasoconstriction[4,5,6].

Its brand name is Migditan and its generic name is Lasmiditan. It was also approved by the European Commission on August 17, 2022. The IUPAC name of Lasmiditan is 2,4,6-trifluoro-N-[6-(1-methylpiperidine-4-carbonyl) pyridin-2-yl] benzamide. The structure of Lasmiditan is shown in the Fig.1.

Lasmiditan is a white, crystalline powder that dissolves in methanol, ethanol, and water.

This compound exhibits a pyridinyl-piperidine scaffold. In a literature survey, a high-performance liquid chromatographic method[7] was reported in which a column of Inertsil Octa Decyl Column dimensions of 150mm x 4.6mm, 3.5µm was reported, and the flow rate used for elution was 1ml/min. This method used 0.1 percent Ortho-phosphoric acid and Acetonitrile in the ratio 50:50 as the mobile phase. In a literature survey, another method is reported in which a High-Performance Liquid Chromatographic equipment is used for estimating the Lasmiditan bulk sample in bulk and its dosage form. In this method, the authors used an Inertial C_{18} column

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of dimensions 150 mm x 4.6 mm and particle size of 3.5µm, and Isocratic elution, a flow rate of 1 ml/min is maintained throughout the run time and the mobile phase used is a mixture of 0.1% Ortho-phosphoric acid and Acetonitrile in 50:50 ratios[8]. In a literature survey one more Author *reported* a method for the separation of process related impurities by liquid chromatographic method. In this method authors can separate five process related impurities of Lasmiditan by using four different columns namely Eclipse-XDB-C_8, Primasil-C_{18}, Inertsil-C_{18}-3V, and Zorbax-SB-Phenyl columns. The mobile phase used for this estimation is 20 mM buffer (Dipotassium hydrogen phosphate) and methanol in different proportions[9]. Altogether, in the literature currently, there is no spectroscopic method for quantifying this drug in bulk samples and their formulation is found. So, the authors developed and validated a simple, precise, economical, and less time-consuming spectrophotometric method to quantify Lasmiditan in bulk drug samples and its formulations.

Table 1. Determination of Lambda max (λ_{max})

Wavelength (nm)	Absorbance
300	0.012
450	0.018
626	0.033
750	0.014
800	0.002

METHODOLOGY

Instruments used

A Shimadzu UV-1700 Pharmaspec model spectrophotometer was used for the spectral and absorbance measurements with a 1cm quartz sample cuvette. All the pH measurements were done Digisun-Digital pH meter.

Stock solutions preparation

A standard drug solution of the Lasmiditan RS was prepared by taking 100 mg of the drug in 100 mL volumetric flask & make up the volume to 100 mL to get 1000mcg/ml (Stock-I) from the above Stock-I solution take 10ml and make up the volume to 100ml to get 100mcg/ml solution (Stock-II).

Preparation of reagents

Preparation of 3-Methyl-2-Benzothiazolinone Hydra zone (0.5%W/V)

0.5 g of Methyl-2-Benzothiazolinon hydrazine hydrochloride (MBTH), was dissolved in 10ml water.

Preparation of 0.2%W/V Ferric Chloride

200 mg of Ferric Chloride anhydrous was dissolved in 100ml distilled water.

Procedures

Estimation of bulk-samples

Calibration Curve

Suitable aliquots (1–5 ml of Lasmiditan Stock–II) were added to a series of 10 ml volumetric flasks, 2ml of Methyl-2-Benzothiazolinon hydrazine hydrochloride (0.5%W/V) and 2.0ml of Ferric chloride (0.2%w/v) were added to each volumetric flask. After heating the resultant solutions for ten minutes at 60°C in a water bath,

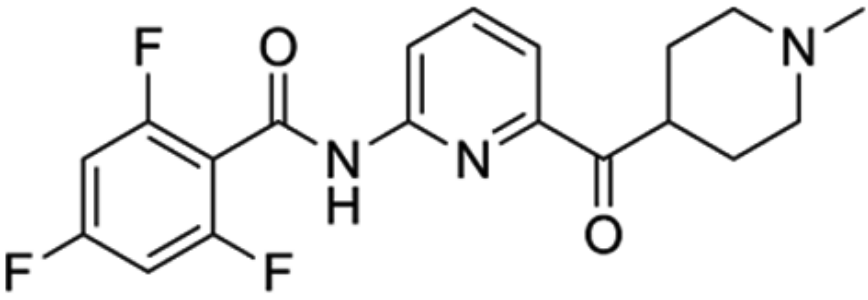

Fig. 1. Structure of Lasmiditan

methanol was added to bring the final volume to 10 milliliters. At 626 nm, the absorbance was measured by using the reagent blank. Plot a regression line by taking absorbance on the Y-axis and concentration on the X-axis.

Bulk sample estimation

A sample from a bulk drug is prepared by dissolving 100mg of bulk drug sample (Active Pharmaceutical Ingredient) in a 100 volumetric flask, dissolving and making up the volume to obtain a stock solution of 1mg/ml. i.e.;1000 ug/mL (Stock-I) from the above Stock-I solution take 10 mL and make up the volume to 100 mL to get 100ug/ml solution (Stock-II). Take 3 mL of the Stock-II solution, 2 ml of Methyl-2-Benzothiazolinon hydrazine hydrochloride (0.5%W/V) and 2.0 mL of Ferric chloride (0.2% w/v) were added to volumetric flask of 10 mL. The volumetric flask is heated on a water bath at 60! for 10min. After that, methanol was then added to make up to 10 mL. At 626 nm, the absorbance was measured by using a reagent blank. Based on

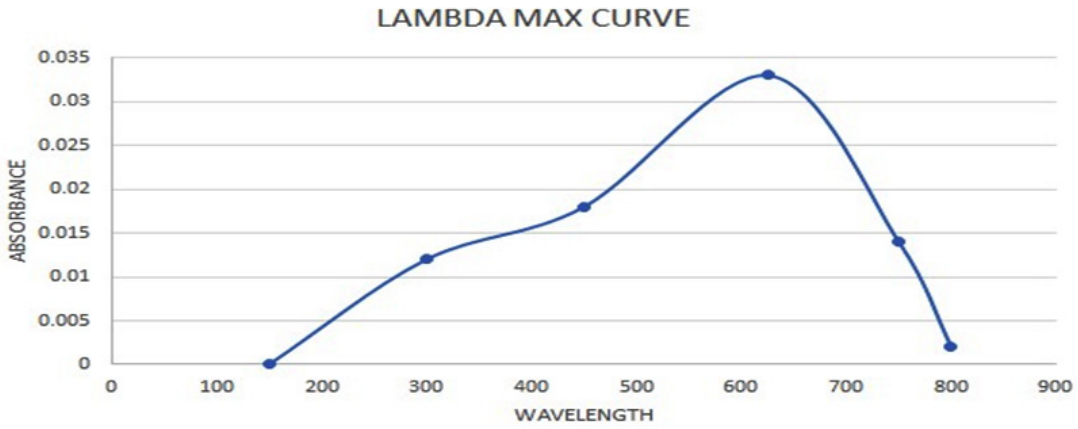

Fig. 2. Absorption spectrum of Lasmiditan

Discussion: Wave maxima(λ max) was found to be 626 nm as the maximum absorbance is seen at particular wavelength

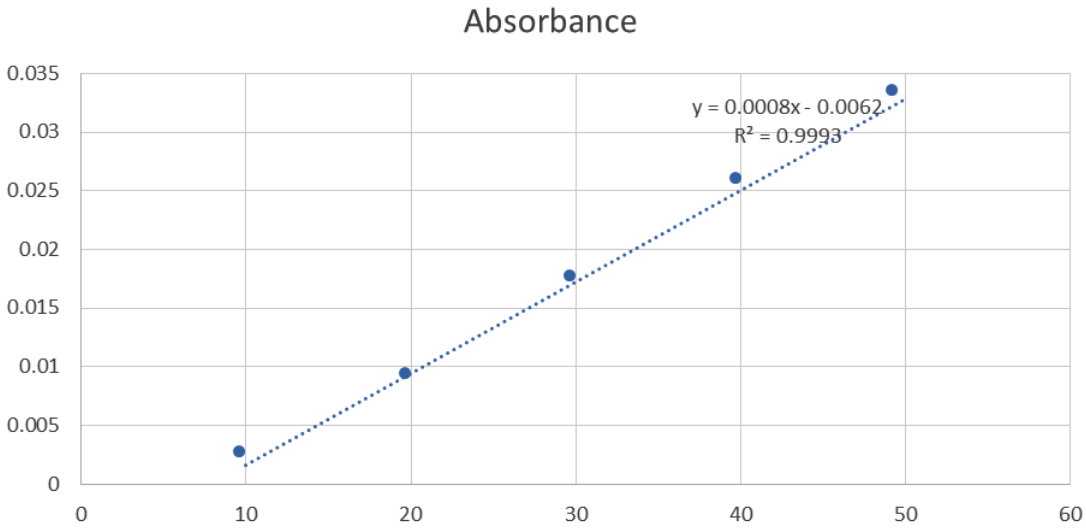

Fig. 3. Linearity plot

Discussion: Concentration(mcg/ml) is taken on X-Axis and the absorbance is taken on Y-Axis, plot is showing linearity with regression co efficient of 0.9993, which is in regulated limit

Table 2. Optical characteristics

01	λ_{max} (nm)	626
02	Regression equation (y = mx + c)	Y=0.0008x-0.0062 Slope (m)= 0.0008 Intercept (c)= -0.0062
03	Beer's law range (mcg/ml)	10-20
04	Regression-co-efficient (R^2)	0.999
05	Precision (%Relative Standard Deviation)	0.128
06	Standard Error of Mean	0.371

Table 3. Absorbance at optimum concentration range

Concentration (mcg/ml)	Absorbance
10	0.002
20	0.008
30	0.017
40	0.022
50	0.033

its calibration curve, the amount of Lasmiditan in the specified bulk drug sample was calculated.

Estimation in formulations

Weigh and powder ten tablet formulations, then take equivalent weight to 100mg of Lasmiditan, was dissolved in 50 mL of methanol, sonicated for 15 minutes, makeup to a final volume of 100ml with Methanol (Stock-I). From the above stock solution, 10 mL was taken and made up to the volume of 100

Table 4. Assay of Tablets

Sample No	Labelled amount (mg/tab)	Amount found (mg/Tab)	% Label claim	% Deviation
1	100	98.00	98	-2.00
2	100	97.50	97.5	-2.50
3	100	96.00	96.00	-4.00

Discussion: Assay results shows that labelled amount and the amount found in the assay is matching with acceptable percentage deviation.

Table 5. Precision results

Serial Number	Concentration (mcg/mL)	Absorbance	Absorbance Average	Standard Deviation	% Relative Standard Deviation
1.	30	0.017	0.0176	0.0014	8.49%
2.	30	0.018			
3.	30	0.016			
4.	30	0.018			
5.	30	0.019			
6	30	0.020			
7	30	0.016			
8	30	0.019			
9	30	0.016			

Discussion: Precision was studies shows that standard deviation and the percentage standard deviation were found to be within regulated limits. The Percentage Relative Standard Deviation was less than 10%

Table 6. Recovery studies

Amount Added (µg/ml)	Amount recovered (µg/ml)	Percentage recovery (%)	Average Recovery	% Relative Standard Deviation
10	09.750	97.50	98.92	1.27%
20	19.970	99.85		
30	29.830	99.43		

Discussion: In recovery studies, percentage recovery, average recovery, and the percentage relative standard deviation were found in regulated limits. The Percentage Relative Standard Deviation was less than 10%

Table 7. Summary

Parameter	Description/Result
Analytical method	Visible Spectrophotometric
Wave length maxima (λ_{max}) in nm	626
Beer's Law range (mcg/ml)	10-50
Regression Plot	Y=0.0008x-0.0062 Slope (m)= 0.0008 Intercept (c)= -0.0062
Regression Coefficient (R^2)	0.9993
% Relative Standard Deviation(Precision)	8.49 %
Molar Absorptivity	$1.303/10^3$
Sandal's Sensitivity (mcg/ml/cm^2/0.001 absorbance Unit)	0.0032
Standard Error of Mean	0.301
Formulation Analyzed	Lasmiditan (MIGDITAN-100MG)
Percentage Recovery	98.92

mL (Stock-II). Taking 3.0 mL of the above solution, 2.0 mL of Methyl-2-Benzothiazolinon hydrazine hydrochloride (0.5%W/V), and 2.0 ml of Ferric chloride (0.2 %w/v) were added to the volumetric flask of 10mL. The volumetric flask is heated in a water bath at 60! for 10min. Next, methanol was added to make up the volume up to 10 ml. At 626 nm, the absorbance was measured relative to a reagent blank. The amount of Lasmiditan in the specified tablet formulation was calculated based on its calibration curve.

RESULTS AND DISCUSSIONS

Method validation

Precision

The precision of the developed method was calculated by taking nine replicates of a fixed quantity of the Lasmiditan [10,11]. The results are indicated in the following Table.5

Recovery studies

Recovery studies were conducted to verify the reliability. A known amount of standard drug was mixed with the pre-analyzed sample formulation, and the contents were then reanalyzed using the suggested approach. The percentage recovery is shown in Table 6.

CONCLUSION

Lasmiditan in tablet formulations and bulk drug forms can be quantitatively estimated accurately by the proposed visible spectrophotometric approach. The method was validated for molar absorptivity, accuracy, precision, and sandal sensitivity. This method is rapid, sensitive, precise, accurate, and efficient. Linearity was observed from 10 to 50 µg/ml concentration range. Average recovery and % relative standard deviation were within the

regulated limits. In precision studies, the standard deviation and the percentage standard deviation were within the regulated ICH limits. The suggested technique can be applied to routine bulk, formulation analysis in quality control laboratories.

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Conflict of Interest

The authors' do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Approval Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Authors' Contribution

The first author had done the method development and validation. The second author Provided the drug samples and reagents being the head of the Institute helped in drafting the manuscript

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