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# Novel high performance liquid chromatographic method development and validation for the estimation of lasmiditan in marketed formulation

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**Abstract**
UV and RP-HPLC method was developed for estimation of Lasmiditan in bulk and tablets dosage form by isocratically using Methanol: Acetonitrile in the ratio of 50:50 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 228 nm. Then developed method was validated by using various parameters. The linearity of analytical method to elicit test results that are proportional to the concentration of analyte in sample within a given range.
Specificity of the method was determined and the peaks of diluent, mobile phase and excipient of tablets did not interfere with standard peaks Lasmiditan. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. The assay value of drugs was close to 100, SD and % RSD are less then 2 indicate the no interferenceof excipient in the estimation of drug.
**Keywords:** UV, RP-HPLC, Lasmiditan, Linearity, Accuracy

## INTRODUCTION

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds [1-3]. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used [4-6]. The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time. Common solvents used include any miscible combinations of water or organic liquids (the most common are methanol and acetonitrile) [7]. Sepration has been done to vary the mobile phase composition during the analysis; this is known as gradient elution. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte.
Liquid chromatographic systems were to an inefficient because of the flow rate of solvents being reliant on gravity. Separations took numerous hours, and some of the time days to finish. Gas chromatography (GC) at the time was more effective than liquid chromatography (LC), in any case, it was trusted that gas stage partition and investigation of extremely polar high atomic weight biopolymers was impossible. GC was ineffectual for some organic chemists due to the thermal instability of the solutes. Accordingly, alternative techniques were hypothesized which would soon bring about the advancement of HPLC [8-12]. Migraine is one of the most common neurological diseases. Migraine presents with severe, intermittent attacks of headache associated with nausea, vomiting, phonophobia and photophobia .It can be chronic ad disabling. It is treated by non specific analgesics, like NSAIDS and specific drugs like triptans and ergot derivatives. The triptans have the risk of life threatening cardiovascular side effects because of their activation of 5 HT1Breceptor [13].
Lasmiditan, sold under the brand name Reyvow, is a medication used for the acute (active but short-term) treatment of migraine 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, dizziness, tiredness, 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, including dizziness and sedation. It should be used with caution iftaken in combination with alcohol or other CNS depressants [14-17].

## 2. MATERIAL AND METHODS

Lasmiditan is an oral 5HT1F agonist used for the acute treatment of migraine headache with or without aura. Lasmiditan, in contrast, is a highly selective agonist of 5-HT1F receptors, carrying virtually no affinity for other receptors which appear to be largely responsible for the adverse effect profile of its predecessors - in other words, lasmiditan's selectivity allows for the

successful termination of migraines without causing vasoconstriction.

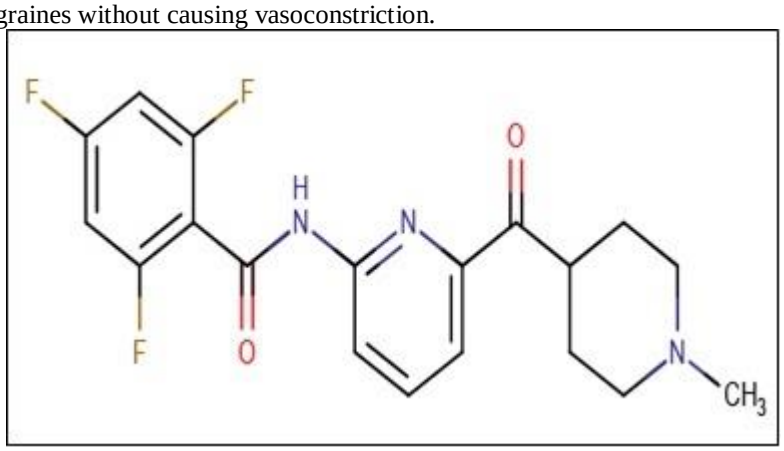



**Figure 1: Structure of Lasmiditan**

**2.1 Characterization and identification of Lasmiditan**

**Physiochemical characteristics Description**- Solid, White to off white powder.

**Melting point**- M.P. of the drug was 196-198ºC found through melting pointapparatus.

**Solubility**- Solubility of Lasmiditan was established by B.P. method.

**2.2 Estimation of Lasmiditan by spectrophotometry**

**2.2.1 Determination of $\lambda_{max}$ of Drug**

Standard solution (10µg/ml) of pure Lasmiditan was prepared. The pure drug solution was scanned on UV spectrophotometer, which showed maximum absorbance at 228.0 nm.

**2.2.2 Experimental Procedure**

10 mg of Lasmiditan was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the water, to give a stock solution of 1000 ppm.

**2.2.3 Preparation of Working Standard Solution**

From stock solutions of Lasmiditan 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with water gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

**2.2.4 Preparation of the Calibration Curves of the Drug**

The standard drug solutions were taken absorbance 3 times and the mean area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve. A typical spectrum and the calibration curve were obtained.

**2.2.5 Preparation of Analysis of Tablet Formulation**

Equivalent to 10mg Lasmiditan was weighed and transferred to a 10 ml volumetric flask and volume was made up to 10 ml with diluents to obtain concentration of 1000µg/ml. Resultant solution was filtered through Whatmann filter paper (No 41). 1 ml of filtrate was taken in 10 ml volumetric flask and volume was made up to 10 ml with diluent to obtain concentration of 100µg/ml. Further 1.0 ml of this solution was taken and diluted up to 10 ml obtain final concentration of 10 µg/ml. Area of the sample solutions at 228.0 nm was measured and from the absorbance values, the concentration of drugs in the sample solution was determined by using Calibration Curve.

**2.3 Validation**

**2.3.1 Linearity**

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to absorbance of analyte in the sample. The calibration plot was constructed after analysis of five different (from 5 to 25µg/ ml) concentrations and absorbances for each concentration were recorded three times, and mean absorbance was calculated. The regression equation and correlation coefficient of curve and the standard curve of the drug is shown in figure.

**2.3.2 Accuracy**

Recovery studies were performed to validate 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.

**2.3.4 Precision Repeatability**

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

**Intermediate Precision**

**Day to Day**

**Analyst to Analyst**

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The

standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

**2.4 LOD (Limit of Detection)**

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula.

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of calibration curve**,** σ = standard deviation of the response.

**2.5 LOQ (Limit of Quantification)**

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula.

$$LOQ = 10 (\sigma / S)$$

Where, S = slope of calibration curve**,** σ = standard deviation of the response.

**2.6 Analytical method development by HPLC**

**2.6.1 Mobile Phase Selection**

Initially to estimate Lasmiditan number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol: Acetonitrile in the ratio of (50:50v/v). The mobile phase was filtered through 0.45 filter paper to remove particulate matter and then degassed bysonication. Flow rate employed for analysis was 1.0 ml/min.

**2.6.2 Selection of wavelength**

100 mg of Lasmiditan was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the methanol. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200- 400nm), maximum absorbance was found at Lambda max 266 nm.

**2.6 3 Selection of Separation Variable**

Standard drug solution of Lasmiditan was prepared in different mobile phase and chromatograph was recorded by using different column (5 and 10 µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

**2.6.4 System Suitability Parameters**

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Lasmiditan10 □g/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

**2.6.5 Preparation of Standard Stock Solution:-**

10 mg of Lasmiditan was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with methanol to give a stock solution of 1000ppm.

**2.6.6 Preparation of Working Standard Solution**

From stock solutions of Lasmiditan1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

**2.6.7 Preparation of the calibration curves of the drug**

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equationwas found out by using this curve.

**2.7 Analysis of tablet formulation**

**2.7.1 Assay of Tablet formulation**

Powder equivalent to 10mg Lasmiditan was weighed and transferred to a 10 ml volumetric flask and volume was made up to 10 ml with methanol to obtain concentration of 1000µg/ml. Resultant solution was filtered through Whatmann filter paper (No. 41). 1 ml of filtrate was taken in 10 ml volumetric flask and volume was made up to 10 ml with diluents (methanol) to obtain concentration of 100µg/ml. Further 1.0 ml of this solution was taken and diluted up to 10ml obtain final concentration of 10 µg/ml. The amounts of Lasmiditanin Tablet formulation was calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with Tablet formulation.

**2.8 Validation**

**2.8.1 Linearity**

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in fig. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

**2.8.2 Accuracy**

Recovery studies were performed to validate 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.

**2.9 Precision**

**Repeatability**

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

**Intermediate Precision**

**Day to Day**

**Analyst to Analyst**

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods. The statistical analysis method was carried out and the data is presented.

**2.10 Robustness**

As per ICH norms, small, but deliberate variations, by altering the pH and / or concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Lasmiditan was studied.

## 3. RESULTS AND DISCUSSION

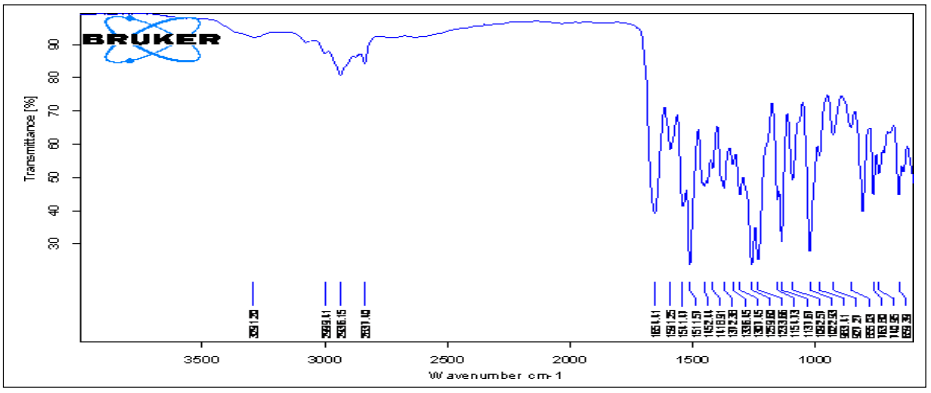
**3.1 Identification and characterization of drug**

**3.2 Melting point**

Melting point of Lasmiditan found to be 196-198ºC.

**3.2 FTIR of Lasmiditan**

**Figure 2: FTIR of Lasmiditan**




**3.3. Solubility study**

**Table 1: Solubility of Lasmiditan**

| S. No. | Solvent | Solubility |
|---|---|---|
| 1 | Water | Soluble |
| 2 | 0.1 N HCl | Soluble |
| 3 | Methanol | Freely Soluble |
| 4 | Ethanol | Freely Soluble |
| 5 | 0.1 N NaOH | Soluble |

**3.4 Determination of $\lambda_{max}$ of Drug**

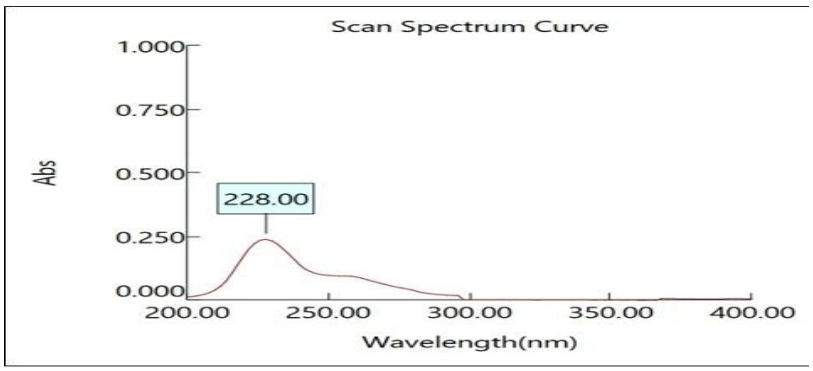



**Figure 3: Selection of $\lambda_{max}$ of Lasmiditan**

### 3.5 Preparation of calibration curves of the drug

**Table 2: Linearity of Lasmiditan**

| | Area | | | | | |
|---|---|---|---|---|---|---|
| **Conc. µg/mL** | **0** | **5** | **10** | **15** | **20** | **25** |
| **Rep.1** | 0 | 15.85 | 30.95 | 46.1 | 61.25 | 75.45 |
| **Rep.2** | 0 | 15.65 | 30.85 | 45.05 | 61.19 | 75.32 |
| **Rep.3** | 0 | 15.74 | 30.69 | 45.09 | 61.18 | 75.43 |
| **Mean** | 0 | 15.747 | 30.830 | 45.413 | 61.207 | 75.400 |
| **S.D.** | 00 | 0.100 | 0.131 | 0.595 | 0.038 | 0.070 |
| **R.S.D%** | 000 | 0.636 | 0.425 | 1.310 | 0.062 | 0.093 |

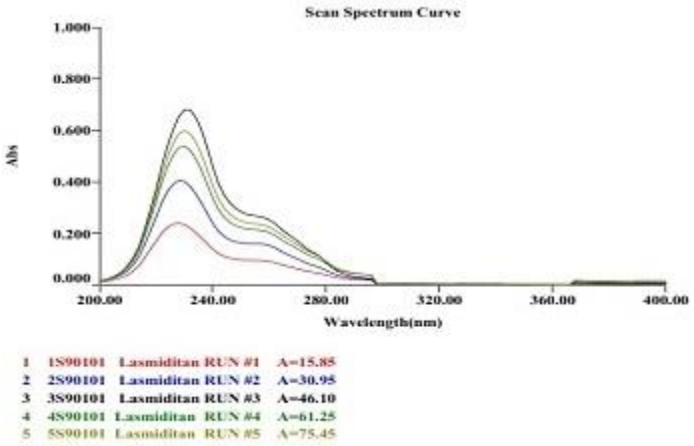



**Figure 3: Linearity of Lasmiditan**

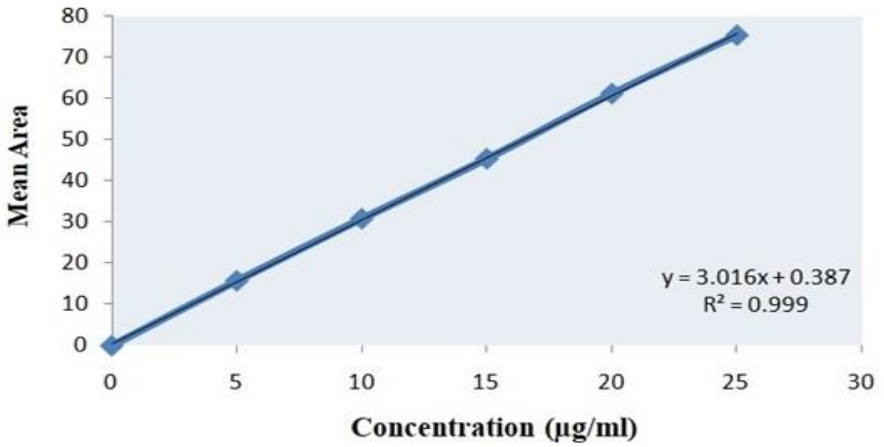



**Figure 4: Calibration Curve of Standard Lasmiditan**

**Table 3: Result of Optical Parameter of Lasmiditan**

| S. No. | Parameters | Observation |
|---|---|---|
| 1. | λ max | 266.0 nm |
| 2. | Beer's law limit (µg/mL) | 5-25 |
| 3. | Regression equation | Y = 3.016x +0.387 |
| 4. | Correlation Coefficient ( $r^2$ ) | 0.999 |

### 3.6 Analysis of Tablet formulation

**Table 4: Assay of Tablet Formulation**

| Brand Name | Lasmiditan | | |
|---|---|---|---|
| | **Label Claim** | **% Found** | **% Purity** |
| **Reyvow-100mg** | 100mg | 99.85mg | 99.85 |

### 3.7 Validation Parameters

### 3.7.1 Accuracy

**Table 5: Recovery Studies for Accuracy of Tablet formulation**

| Level of recovery (%) | 80 | 100 | 120 |
|---|---|---|---|
| Amount Present | 10 | 10 | 10 |
| | 10 | 10 | 10 |
| | 10 | 10 | 10 |
| Amount of std. added | 8 | 10 | 12 |
| | 8 | 10 | 12 |
| | 8 | 10 | 12 |
| Amount Recovered | 7.98 | 9.95 | 11.85 |
| | 7.85 | 9.95 | 11.65 |
| | 7.92 | 9.92 | 11.92 |
| % Recovery | 99.75 | 99.50 | 98.75 |
| | 98.13 | 99.50 | 97.08 |
| | 99.00 | 99.20 | 99.33 |

**Table 6: Statistical Validation of Recovery Studies**

| Level of Recovery (%) | % Recovery | Standard Deviation* | % RSD |
|---|---|---|---|
| 80 | 98.96 | 0.813 | 0.822 |
| 100 | 99.40 | 0.173 | 0.174 |
| 120 | 98.39 | 1.168 | 1.187 |

***Denotes average of three determinations**

### 3.8 Precision

### 3.8.1 Repeatability

**Table 7: Results of analysis Data of Tablet Formulation**

| Drug | Label claim | Amount found* | Label claim (%) | S.D. | % RSD |
|---|---|---|---|---|---|
| **Lasmiditan** | 100mg | 99.12 | 99.12 | 0.154 | 0.165 |

### 3.9 Intermediate Precision (Inter-day and Intra-day precision)

**Table 8: Intra-day and Inter-day precision**

| Intra-day Precision | | Inter-day Precision | |
|---|---|---|---|
| | % Label Claim | | % Label Claim |
| After 1hr | 99.45 | First day | 99.15 |
| After2hr | 99.15 | Second day | 98.85 |
| After3hr | 99.05 | Third day | 98.15 |
| **Mean** | 99.217 | **Mean** | 98.717 |
| **SD** | 0.170 | **SD** | 0.513 |
| **% RSD** | 0.171 | **% RSD** | 0.520 |

### 3.10 Analyst to Analyst

**Table 9: Result of Analyst to Analyst Precision**

| Analyst | Label claim (mg) | Amount found* (mg) | Label claim (%) | S.D. | % RSD |
|---|---|---|---|---|---|
| **1.** | 100 | 99.95 | 99.95 | 0.021 | 0.035 |
| **2.** | 100 | 99.78 | 99.78 | 0.026 | 0.054 |

### 3.11 LOD and LOQ

**Table 10: Results of LOD and LOQ**

| S. No. | Parameter | Results |
|---|---|---|
| 1. | LOD | 0.25µg/ml |
| 2. | LOQ | 0.75µg/ml |

### 3.12 HPLC method

**Mobile Phase Selection**

**Table 11: Mobile Phase selection**

| Mobile phase | Ratio (v/v) | Flow rate | Observation |
|---|---|---|---|
| Water: Methanol | 50:50 | 1.0ml/min | Poor resolution |
| Methanol: Acetonitrile | 50:50 | 1.0ml/min | Most Suitable |

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol: Acetonitrile (50:50v/v) in the ratio of 50:50. The mobile phase was filtered through 0.45□ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

### 3.13 Selection of Separation Variable

**Table 12: Selection of Separation Variable**

| Variable | Condition |
|---|---|
| **Column** | |
| Dimension. | 250mm x 4.60mm |
| Particle Size | 5 |
| Bonded Phase | Octadecylsilane (C18) |
| **Mobile Phase** | |
| Methanol | 50 |
| CAN | 50 |
| Flow rate | 1ml/min |
| Temperature | Room temp. |
| Sample Size | 20 l |
| Detection wavelength | 228.0 nm |
| Retention time<br><br>Lasmiditan | 2.715± 0.3 min |

### 3.14 System Suitability Parameters

**Table 13: Result of System Suitability Parameters for Lasmiditan**

| System suitability Parameter | RT | AUC | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| **Rep-1** | 2.821 | 505.687 | 2545 | 1.12 |
| **Rep-2** | 2.789 | 510.256 | 2565 | 1.15 |
| **Rep-3** | 2.791 | 501.325 | 2540 | 1.11 |
| **Mean** | 2.800 | 505.756 | 2550.000 | 1.127 |
| **S.D.** | 0.018 | 4.466 | 13.229 | 0.021 |
| **% RSD** | 0.640 | 0.883 | 0.519 | 1.848 |

### 3.15 Linearity and Calibration Graph

**Table 14: Result of Linearity of Lasmiditan**

| Std. Conc. g/ml | 5 | 10 | 15 | 20 | 25 |
|---|---|---|---|---|---|
| **1** | 256.125 | 505.687 | 756.658 | 1010.37 | 1225.66 |
| **2** | 265.856 | 510.256 | 750.236 | 1005.66 | 1220.37 |
| **3** | 245.589 | 501.325 | 748.854 | 1008.85 | 1215.75 |
| **Mean** | 255.857 | 505.756 | 751.916 | 1008.29 | 1220.59 |
| **SD** | 10.136 | 4.466 | 4.164 | 2.403 | 4.960 |
| **%RSD** | 3.962 | 0.883 | 0.554 | 0.238 | 0.406 |

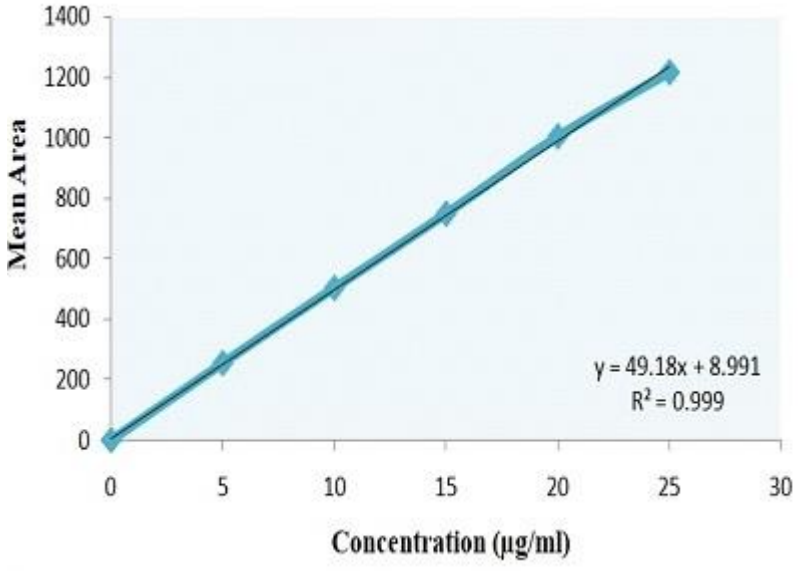



**Figure 5: Calibration Graph of Lasmiditan**

Regression Equation Y=mx+c,
Y= AUC
m= slope = 49.18 X= Conc. in µg/ml c= Intercept = 8.991 $r^2$=0.999

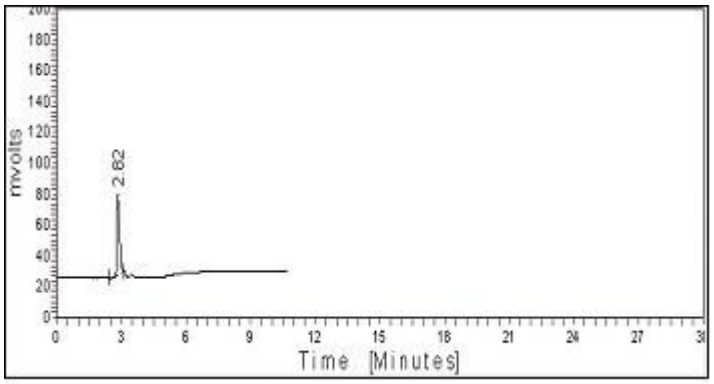



**Figure 6: Chromatogram of Lasmiditan 5ppm**

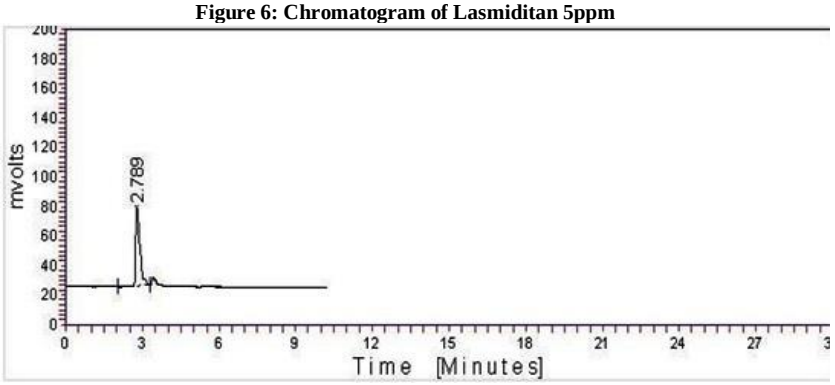



**Figure 7: Chromatogram of Lasmiditan 10ppm**

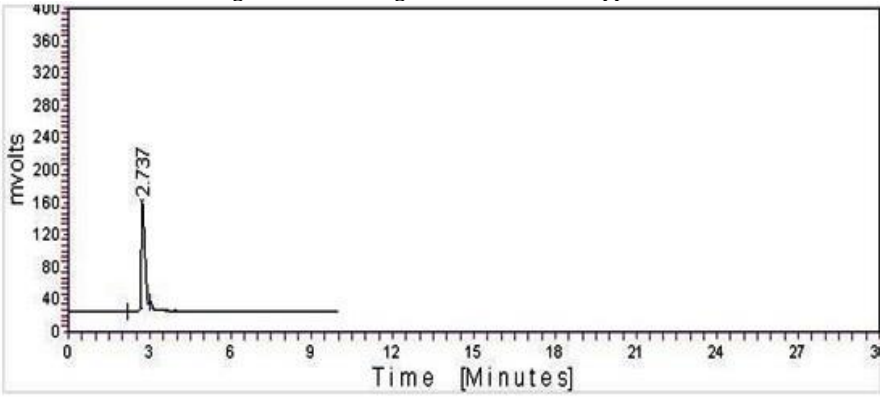



**Figure 8: Chromatogram of Lasmiditan 15ppm**

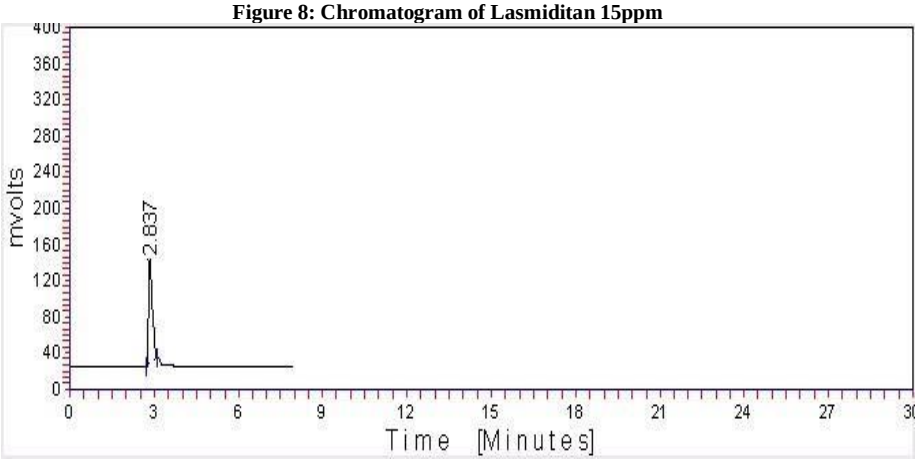



**Figure 9: Chromatogram of Lasmiditan 20ppm**

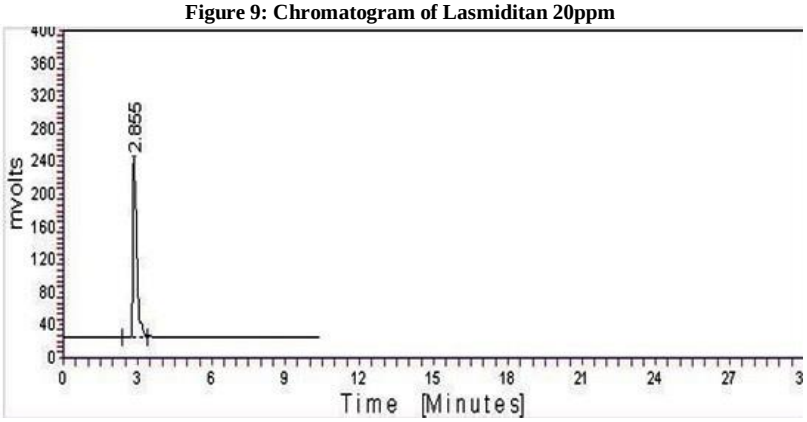



**Figure 10: Chromatogram of Lasmiditan 25 ppm**

### 3.17 Assay of Tablet Formulation

**Table 15: Result of analysis for Lasmiditanin tabltets formulation**

| Std Conc. µg/ml | LASMIDITAN | | |
|---|---|---|---|
| | 5 | 10 | 15 |
| Rep-1 | 4.98 | 9.98 | 14.95 |
| Rep-2 | 4.95 | 9.99 | 14.98 |
| Rep-3 | 5.01 | 10.01 | 14.95 |
| Rep-1 | 99.60 | 99.80 | 99.67 |
| Rep-2 | 99.40 | 100.10 | 100.20 |
| Rep-3 | 101.21 | 100.20 | 99.80 |
| Mean | 100.070 | 100.033 | 99.889 |
| SD | 0.994 | 0.208 | 0.278 |
| % RSD | 0.994 | 0.208 | 0.278 |

***Each reading is mean reading of three batch of formulation**

### 3.18 Validation of developed Method

#### 3.18.1 Linearity

**Table 16: Response Ration Data for Linearity of Lasmiditan**

| Replicates | Concentration (□g/ml) | Mean AUC | Response Ratio |
|---|---|---|---|
| Rep-1 | 5 | 255.8567 | 51.17 |
| Rep-2 | 10 | 505.756 | 50.58 |
| Rep-3 | 15 | 751.916 | 50.13 |
| Rep-4 | 20 | 1008.292 | 50.41 |
| Rep-5 | 25 | 1220.589 | 48.82 |
| Mean | | | 50.22 |
| S.D. | | | 0.870 |
| % R.S.D. | | | 1.732 |

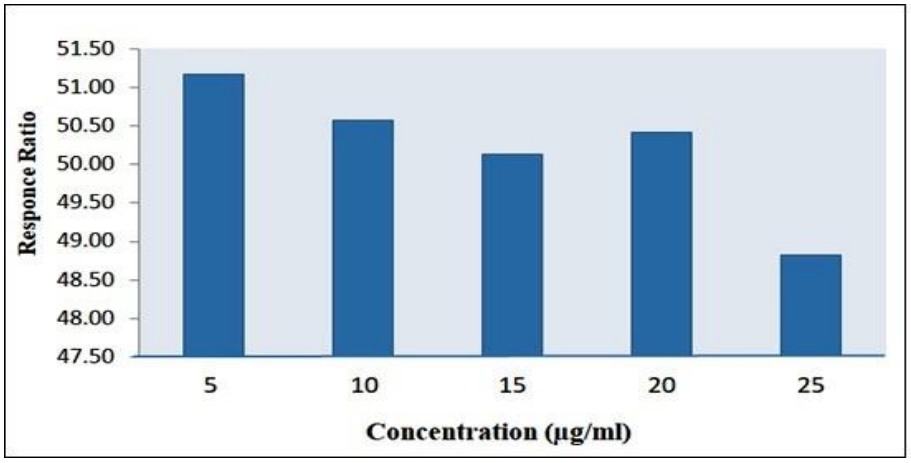



**Figure 11: Response Ratio Curve of Lasmiditan**

#### 3.18.2 Accuracy

**Table 17: Recovery Studies of Formulation**

| Level of Recovery (%) | 80 | 100 | 120 |
|---|---|---|---|
| Amount present(mg) | 5<br>5<br>5 | 5<br>5<br>5 | 5<br>5<br>5 |
| Amount of Std.added (mg) | 4<br>4<br>4 | 5<br>5<br>5 | 6<br>6<br>6 |
| Amount recovered (mg) | 102.50<br>100.00<br>99.50 | 4.9<br>5.1<br>5.1 | 6.0<br>6.1<br>5.9 |
| % Recovery | 100.667<br>1.607<br>1.597 | 98.00<br>102.00<br>102.00 | 100.00<br>101.67<br>98.33 |

**Table 18: Statistical Validation of Recovery Studies**

| Level of Recovery (%) | % Recovery | Standard Deviation* | % RSD |
|---|---|---|---|
| 80 | 100.66 | 1.607 | 1.597 |
| 100 | 100.66 | 1.989 | 1.954 |
| 120 | 100.00 | 1.667 | 1.667 |

***Denotes average of three determinations**

### 3.18.3 Precision

**Repeatability**

**Table 19: Results of analysis Data of Physical Mixture**

| Drug | Label claim | Amountfound* | Labelclaim (%) | S.D. | % RSD |
|---|---|---|---|---|---|
| **Lasmiditan** | 100mg | 99.95 mg | 99.95 | 0.615 | 0.645 |

### 3.18.4 Intermediate Precision- (Inter-day and Intra-day Precision)

**Table 20: Intra-day and Inter-day Precision**

| Intra-day Precision | | Inter-day Precision | |
|---|---|---|---|
| | % Label Claim | | % Label Claim |
| After 1hr | 99.90 | First day | 98.00 |
| After 2hr | 99.50 | Second day | 97.50 |
| After 3hr | 99.20 | Third day | 97.00 |
| After 4hr | 99.00 | | |
| After 5hr | 98.90 | | |
| After 6hr | 98.30 | | |
| **Mean** | 99.13 | **Mean** | 97.50 |
| **SD** | 0.546 | **SD** | 0.500 |
| **% RSD** | 0.551 | **% RSD** | 0.512 |

### 3.18.5 Analyst to Analyst

**Table 21: Analyst to Analyst**

| Analyst | Label claim (mg) | Amount found* | Label claim (%) | S.D. | % RSD |
|---|---|---|---|---|---|
| **1** | 100 | 99.95 | 99.95 | 0.110 | 0.158 |
| **2** | 100 | 99.45 | 99.45 | 0.225 | 0.159 |

### 3.18.6 Robustness

**Table 22: Result of Robustness of Formulation**

| Compound | % RSD in Normal | Changed Condition n= 6 | |
|---|---|---|---|
| Temperature | | - 5 °C | + 5 °C |
| **Lasmiditan** | 0.34 | 0.67 | 0.58 |
| Flow rate | | (-10%) | (+10%) |
| **Lasmiditan** | 0.49 | 0.79 | 0.99 |
| Mobile phase ratio | | - 2 % | + 2 % |
| **Lasmiditan** | 0.34 | 0.85 | 0.90 |

## SUMMARY AND CONCLUSION

The U.V and RP-HPLC method was developed for estimation of Lasmiditan in bulk and tablets dosage form by isocratically using Methanol: Acetonitrile in the ratio of 50:50 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 228 nm. Then developed method was validated by using various parameters. The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 µg/ml of Lasmiditan were injected separately and chromatogram was recorded. The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. Specificity of the method was determined and the peaks of diluent, mobile phase and excipient of tablets did not interfere with standard peaks Lasmiditan. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less then 2 indicate the precision of method. The robustness of developed method was checked by changing in the deliberate variation in solvent. The results of the analysis of tablet formulation were reported. The assay value of drugs was close to 100, SD and % RSD are less then 2 indicate the no interferenceof excipient in the estimation of drug.

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