Development and validation of RP-HPLC method of Lafutidine (API)

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Abstract

The goal of this study is to develop and validate an RP HPLC technique for estimating Lafutidine in bulk form. A thorough review of the literature found that there are ways available, but none of them are as straightforward, specific, exact, or accurate as the established approach for estimating Lafutidine. The chromatographic method was found to be suitable for effective separation of Lafutidine with good resolution, peak shape. The mobile phase composed of 0.1M ammonium acetate buffer (pH 7.5): Methanol (80:20 v/v), at a flow rate of 1.4 ml/min was selected as it gave well resolved peaks of standard Lafutidine. The optimum wavelength 290nm selected for detection and quantitation. According to ICH criteria, the devised approach was validated.

Key Words: RP-HPLC, Method development, Validation, Lafutidine

DOI Number: 10.14704/nq.2021.19.11.NQ21237 **NeuroQuantology 2022; 19(11):346-355**

Introduction

Lafutidine is Chemically 2-(furanylmethylsulphinyl)-N-[(Z)-4-[4- (piperidinyl-methyl)-pyridin-2-yl) oxybut-2-enyl]acetamide.It is a second generation histamine receptor antagonist (H2-RA) possessing an antisecretory effect as well as gastro protective activity against several necrotizing agents independent of its antisecretory action.[1-3]

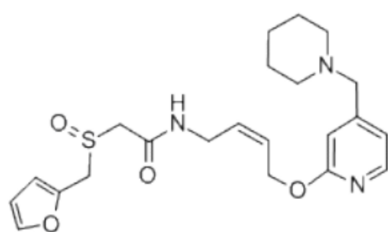

Figure 1: Structure of Lafutidine

Literature survey reveals that, there are several spectroscopic, FTIR5, HPLC, HPTLC and LC-MS. methods for the estimation of Lafutidine[3-5] There are few UV and HPLC methods are reported of Lafutidine in their combined dosage form. So that need was felt, to develop new methods to analyze the drugs simultaneously. A successful attempt has been made to estimate two drugs simultaneously by RP-HPLC method. [5-9] The present work demonstrates simple, rapid, accurate, reproducible and economical method for the Lafutidine.

Materials And Methods

Chemicals And Reagents

Lafutidine was obtained from Pure Chem Pvt. Ltd., Gujrat, India. All the chemicals used were of analytical grade and HPLC grade procured from Qualigens, India Ltd.

Methods

Preformulation Studies

1. Organoleptic properties

The sample of Lafutidine was studied for organoleptic characters such as color, odor and appearance.

2. Melting point

The melting point of lafutidine was found using melting point apparatus. For the purpose of determining the m.p., the drug was injected into a glass capillary with a flame-sealed aperture.

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The capillary containing the drug was submerged in liquid paraffin inside the magnetic stirring m.p. apparatus.

3. FTIR Spectroscopy

Lafutidine dry sample and IR grade KBr were mixed at a ratio of 1:100. This mixture was compressed into a pellet by applying 10 tonnes of pressure with a hydraulic press. A wave number range of 4000 to 400 cm-1 was used to scan the pellets. An FTIR instrument was used to conduct the spectral study.

4. Differential scanning calorimetry studies

The thermal examination was carried out using the Mettler Toledo DSC-823'e system, which includes a differential scanning calorimeter and a computerized data station. A nitrogen flow was applied while weighed samples of pure medicines, physical mixes of drugs and polymers, and mixtures of polymers were heated at a rate of 10°C/min between 40 and 200°C. Differential scanning calorimetry research sheds light on how various materials interact with one another at various temperatures.

5. Physical compatibility test

A preformulation study employing potential formulation polymers was done to determine the interaction/compatibility between the drug and the polymer. In a 1:1 ratio, lafutidine and xyloglucan were equally distributed. The combination was stored in glass vials that were kept at room temperature. Utilizing the aforementioned differential scanning calorimetry and infrared spectroscopy, samples were examined for compatibility after 15 days.

6. Determination of solubility

In solutions of 0.1 N HCl, pH 4.6 acetate buffer, and pH 6.8 phosphate buffer, the solubility of lafutidine was measured. Extra Lafutidine was added to 25 ml of each medium after all the media had been prepared, and the mixture was then placed in a 50 ml conical flask and agitated on a mechanical shaker for 48 hours. 1 cc of each sample was extracted after 48 hours of shaking and placed through Whatman filter paper No 41. Calculations of solubility were done after measuring absorbance in the 200–400 nm range using a UV–visible spectrophotometer.

7. UV Spectroscopy (Determination of λ_{max})

Lafutidine stock solution (100 g/ml) was made in 0.1 HCL. To achieve a concentration of 100 g/ml Lafutidine in 0.1 N HCl and water, respectively, this solution was properly diluted with 0.1 N HCl separately. The UV visible spectrophotometer captured the UV spectrum in the 200–400 nm range.

8. Calibration curve of Lafutidine in 0.1 N HCl.

Following careful weighing, lafutidine (10 mg) was added to a volumetric flask with a 100 ml capacity. It was next dissolved in 25 ml of 0.1 N HCl and then diluted with the same solution to a final volume of 100 ml. To attain a concentration of between 5 and 30 g/ml, the aforementioned solution was further diluted. The absorbance of the resulting solutions was measured at 290 nm using a UV Visible Spectrophotometer. HCl (0.1N) was used as the standard for the blank. After creating calibration plots, the linearity was determined. Three runs of the calibration curve were made.

Analytical method development

Instrumentation and Chromatographic Conditions

The HPLC system employed has a JASCO UV-4075 UV-VIS detector, a model PU 4180 RHPLC pump, a Rheodyne sample injection port (20 l), and ChromNAV CFR chromatography software (version 2.0). HiQSil C18 (250 mm 4.6 mm, 5 m) column separation was performed using 0.1 M ammonium acetate buffer (pH 7.5): Methanol (80:20 v/v) as the mobile phase and 1.4 mL/min as the flow rate. Using a Rheodyne injector with a 20-L loop, samples were injected. Detection was done at 290 nm. Using a Shimadzu balance (Model AY-120), all weighing was done.

Determination of Lambda maximum

Cost, analysis time, assay sensitivity, and solvent noise were taken into consideration while determining if a particular solvent system was appropriate for the analysis of lafutidine. Methanol (80:20 v/v) and 0.1M ammonium acetate buffer (pH 7.5) made up the mobile phase.
Before being injected into the HPLC equipment, methanol and ammonium acetate buffer were first filtered under vacuum using 0.22 m membrane filters and degassed using a sonicator.

Preparation of stock solution of Lafutidine

By combining 50 mg of lafutidine with 20 ml of mobile phase and mixing the mixture, a standard stock solution (1000 g/ml) was created. The mobile phase was then used to fill the remaining space to the mark of 50 ml. The stock solutions were degassed using a sonicator and then filtered through a membrane filter with a 0.22 m pore size.
Lafutidine's lambda maximum was discovered to be 290 nm.

Preparation of mobile phase

Methanol (80:20 v/v) and 0.1M ammonium acetate buffer (pH 7.5) were combined to create the mobile phase. For 30 minutes, gases were removed in an ultrasonic water bath. filtered the mixture using a 0.45-inch filter.

Diluent preparation

Mobile phase used as diluents.

Preparation of standard stock solution

In order to get 1000 g/mL, 25 mg of lafutidine standard were put into a 50 ml volumetric flask, dissolved, and made up to volume with mobile phase. Transferring 1 ml of the aforementioned solution into a 10 ml volumetric flask, making up the volume to volume with mobile phase, and then performing the additional dilutions were done.

Preparation of test solution

Transferred into a 50 ml volumetric flask, the 25 mg equivalent of the lafutidine API standard was dissolved and brought up to volume with mobile phase 1000 g/mL. Transferring 1 ml of the aforementioned solution into a 10 ml volumetric flask, making up the volume to volume with mobile phase, and then performing the additional dilutions were done.

Selection of analytical wavelength

The property of a compound is what contributes to the analyte's or compound's electronic structure. Lafutidine's structural analysis was performed using the standard solution and UV light with a wavelength of 200–400 nm.

Method Validation

Linearity

The new method's linearity was examined for concentration ranges between 5 and 30 g/ml. The standard stock solutions of 0.5, 1, 1.5, 2.0, 2.5, and 3.0 ml were diluted with mobile phase to create the aliquots of 5, 10, 15, 20, 25 and 30 g/ml. The chromatographic apparatus was fed with the acquired concentrations. Lafutidine calibration curve was created by graphing peak area versus utilized concentration. The regression equation and correlation coefficient were assessed to confirm that the concentration range under study is linear.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Lafutidine, a known amount of standard drug powder of Lafutidine was added to 80, 100, 120% level.

Precision method

The accuracy of the approach was evaluated by looking at variations in the intra-day and inter-day periods. In the intra-day experiments, the percent RSD was estimated after six repeated injections of the standard solution. For the inter-day variation studies, %RSD was calculated after six repeated injections of the standard solution over the course of six days.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

Result & Discussion

Preformulation studies

Table 1. Melting point & Organoleptic properties of Lafutidine.

Drug	Melting Point range		Organoleptic properties	
	Literature	Practical	Colour	State
Lafutidine	98-101°C	98-100°C	white to off–white	crystalline powder

Fourier transform infrared spectroscopic (FTIR) studies

The FTIR spectrum of Lafutidine is shown in Figure below and interpretation of FTIR spectra is given in Table. FTIR spectrum of Lafutidine showed all the peaks corresponding to the functional groups present in the structure of Lafutidine.

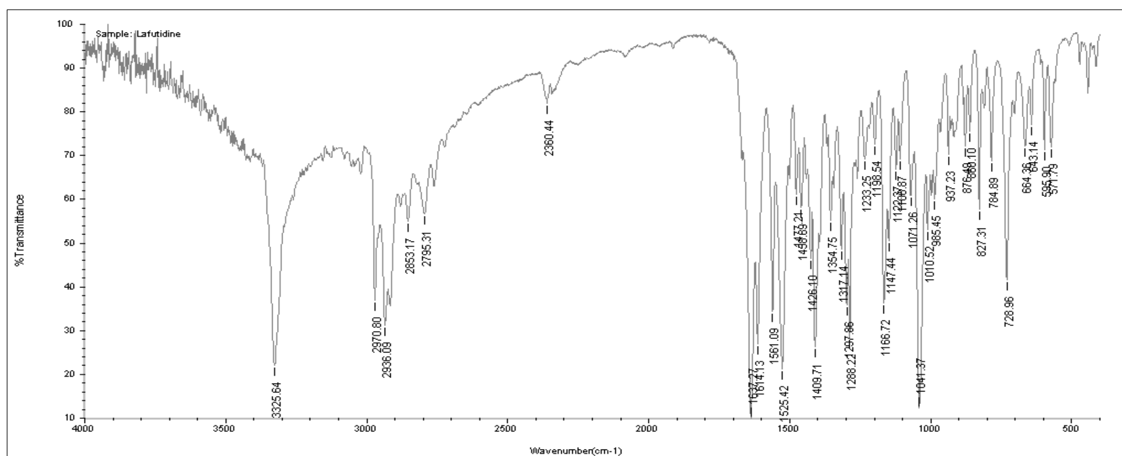

Figure 1: FTIR spectrum of Lafutidine.

The major peaks for pure Lafutidine were seen as below, 3325.64 for–NH stretching, 2360.44 for–CH (alkyl) stretching, 1632.24 for-C=O stretching, 937.23 for SO stretching which confirms the important functional group Lafutidine.

Differential Scanning Calorimetric (DSC) studies

Differential Scanning Calorimetry studies indicated a sharp endothermic peak at 99.21°C corresponding to melting of pure Lafutidine is as shown in Figure.

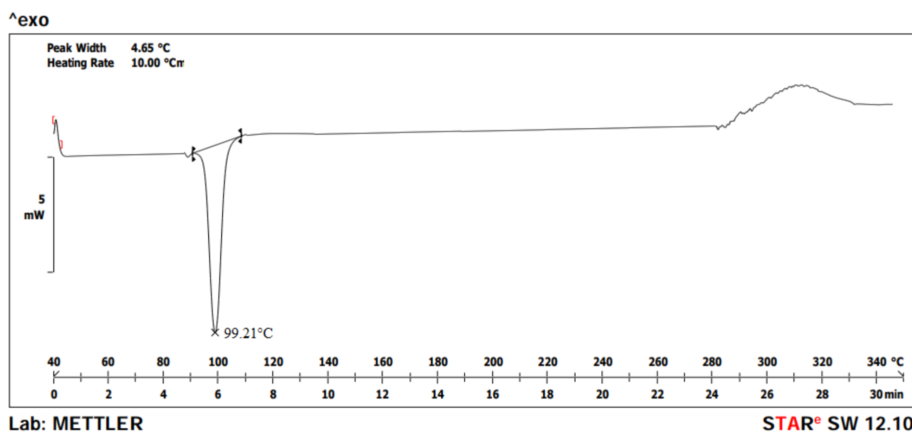

Figure 2. DSC thermogram of Lafutidine.

Physical compatibility test

Physical compatibility test using Infrared Spectroscopy

For physical compatibility test FTIR of drug and excipients were mixed and kept strictly for 30 days. The spectrum was scanned over a frequency range 4000-400 cm-1. FTIR spectra of drug-excipient

mixtures retained the characteristic functional peaks of the drug as shown in Figures below. Thus, the polymer and the drug show no interaction.

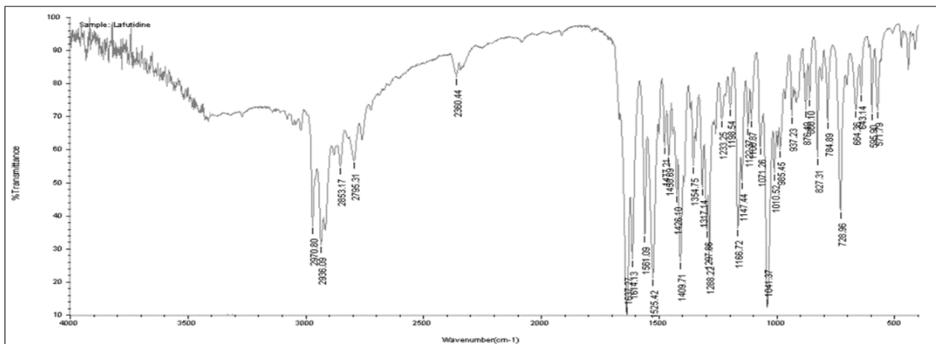

Figure 3. IR spectrum of Lafutidine + Xyloglucan + HPMC K750PH physical Mixture.

The major peaks for pure Lafutidine were seen as below, 2360.44 for–CH (alkyl) stretching, 1632.24 for-C=O stretching, 937.23 for SO stretching which confirms the important functional group Lafutidine.

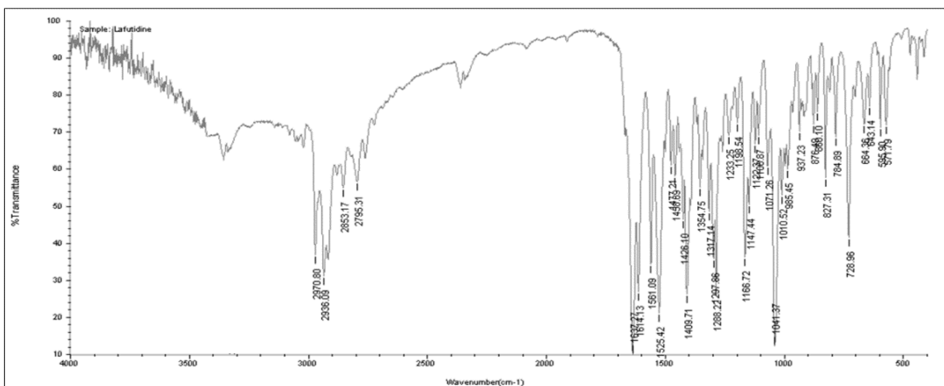

Figure 4: IR spectrum of Lafutidine + gellan gum + HPMC K100 M physical mixture.

The three main peaks for pure Lafutidine were seen as follows: 2360.44 for-CH (alkyl) stretching, 1632.24 for-C=O stretching, and 937.23 for SO stretching, confirming Lafutidine as an essential functional group.

There is no interaction between the drug and excipients, according to the FTIR spectra of pure Lafutidine and physical mixtures, which demonstrate the vibration of functional groups found in the drug's structure.

Physical compatibility test using Differential Scanning Calorimetric (DSC) studies

The Figure displays the DSC thermograms of both the pure medication and its physical mixes with different polymers. Comparative Scanning Studies using a calorimeter showed a pronounced endothermic peak at 99.21°C, which corresponded to the melting point of pure lafutidine. Dilution of the drug in physical mixes of the drug with both polymers led to the observed widening of the peaks as well as changes in relative intensities. It might be said that there is no interaction between the medicine and the polymers. The location of the endothermic peaks prevented the medicine from forming a compound with the excipients.

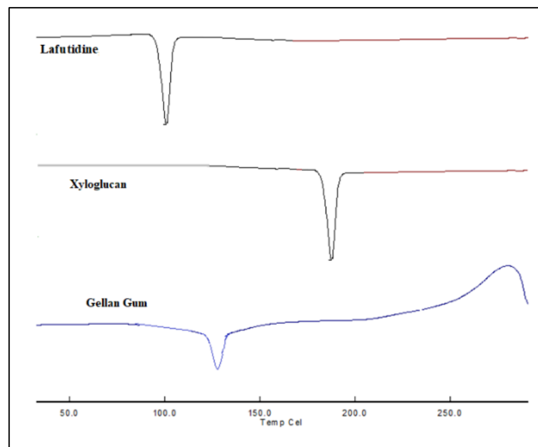

Figure 5: DSC thermogram of Lafutidine, Xyloglucan and gellan gum

From the above observation from FTIR and DSC study, it was concluded that the polymer and drug did not interact with each other and are compatible.

Determination of solubility of Lafutidine

The solubility of Lafutidine as observed in 0.1 N HCl (pH 1.2) and buffers of pH values 4.6 (acetate buffer) and 6.8 (phosphate buffer) are presented in Table. Lafutidine exhibited a pH dependent solubility in these aqueous buffers. Higher solubility of Lafutidine was observed at acidic pH values, while the solubility dropped rapidly as the pH increased (Figure).

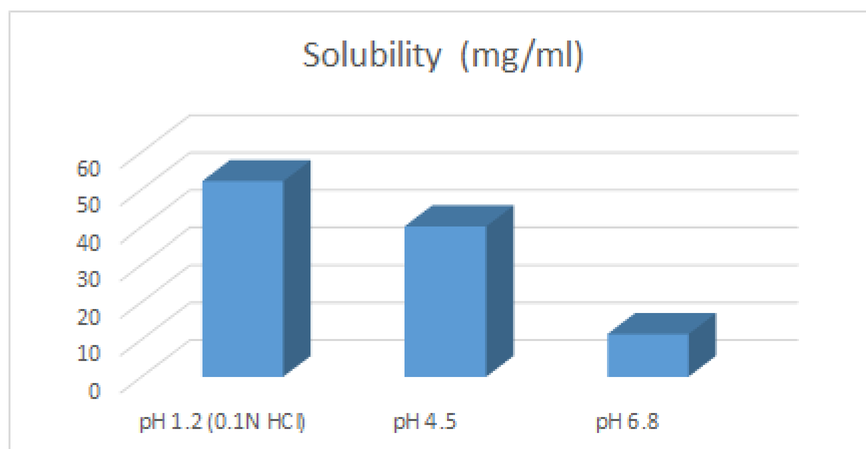

Figure 6: Solubility analysis of Lafutidine.

UV-VIS Spectrophotometric method for Lafutidine

Determination of λmax

Wavelength of maximum absorbance (λ_{max}) of Lafutidine was found to be 290 nm in 0.1 N HCl.

Calibration curve for Lafutidine

The calibration curve for Lafutidine in 0.1 N HCl is shown in Figure 25. The graph of absorbance vs. concentration for Lafutidine was found to be linear in the concentration range of 5-30 μg/ml at 290 nm. The r2 of the calibration curve was found to be 0.9996. The standard calibration curve detail for Lafutidine is shown in Table.

Table 2: Concentration and absorbance values for Lafutidine in 0.1 N HCl.

Concentration (µg/ml)	Absorbance
05	0.0124
10	0.024
15	0.0367
20	0.0485
25	0.062
30	0.0745

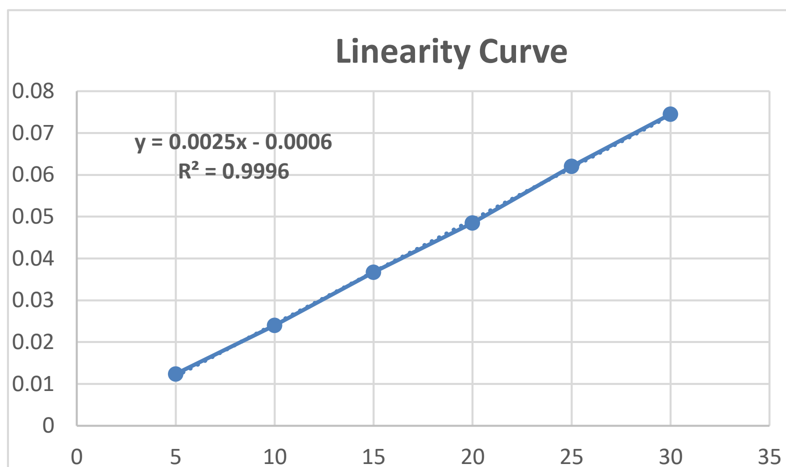

Figure 7: Calibration cure of Lafutidine in 0.1 N HCl.

HPLC analytical method

Selection of wavelength maxima

The solution of Lafutidine was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 290nm.

Method development

The proposed chromatographic method was found to be suitable for effective separation of Lafutidine with good resolution, peak shape given in the figure. The mobile phase composed of 0.1M ammonium acetate buffer (pH 7.5): Methanol (80:20 v/v), at a flow rate of 1.4 ml/min was selected as it gave well resolved peaks of standard Lafutidine. The optimum wavelength 290nm selected for detection and quantitation.

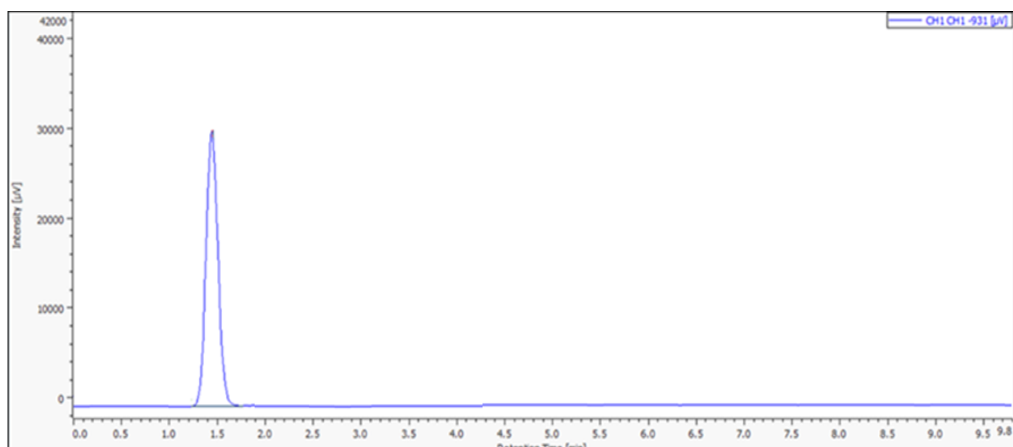

Figure 8: HPLC chromatogram of standard Lafutidine.

The retention time was found to be 1.31 with distinct peak.

Method validation

Linearity

The calibration curves were found be linear for the concentration range of 5-30ppm. The standard working curve equation for drug was found to be y = 3014.2x + 59981 with correlation coefficient value r^2 = 0.9997. The results of linearity are given in Figure below.

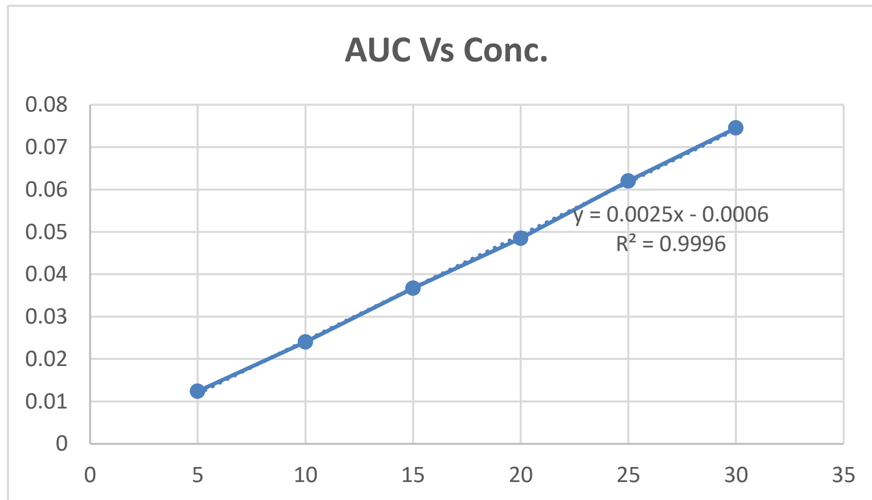

Figure 9: Linearity curve of standard Lafutidine

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Lafutidine given in Table.

Table 3: Recovery data of Lafutidine

Level (%)	Drug Conc. (mg)	Amt. recovered (mg)	% Recovery
80%	8	8.05	100.63
100%	10	9.98	99.8
120%	12	12.20	101.67

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The results of precision studies are shown in Table.

Table 4: Precision study (intra- day) of Lafutidine

Conc µg/mL	Area	AVG	%RSD
10	90215	90202.333	0.05566694
	90147		
	90245		
15	105421	105663.33	0.21482772
	105871		
	105698		
20	120457	120386	0.17102953
	120154		
	120547		

Conc., Concentration; AVG, average; RSD, Relative standard deviation

Table 5: Precision study (inter-day) of Lafutidine

Conc µg/mL	Area	AVG	%RSD
10	90785	90399	0.37550471
	90265		
	90147		
15	105478	105550	0.12144705
	105698		
	105474		
20	120546	120453	0.07411128
	120445		
	120368		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

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

This data showed that the sensitivity of method to determine the drug Lafutidine. The Minimum concentration level at which the analyte can be reliable detected (LOD) &quantified (LOQ) were found to be 1.45 & 1.87 µg/m respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Lafutidine by changing flow rate 1.2 mL/min and 1.6 mL/min, mobile phase composition Methanol: water ratio 79:21 and 81:19, wavelength i.e. 289nm and 291nm. The method was found to be robust in the range of deliberate changes made.

Table 6: Robustness study with change in flow rate of Lafutidine

Flow rate mL/min	Conc. µg/mL	Area	AVG	%RSD
1.2	20	120451	120496	0.04909
1.2		120563		
1.2		120474		
1.6	20	120458	120452.7	0.0084
1.6		120459		
1.6		120441		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 7: Robustness study with change in concentration of mobile phase of Lafutidine

Mobile phase	Conc µg/mL	Area	AVG	%RSD
79:21	20	120236	120379.7	0.13029
79:21		120547		
79:21		120356		
81:19	20	120454	120422.7	0.06312
81:19		120478		
81:19		120336		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table 8: Robustness study with change in Wavelength of Lafutidine.

Wavelength nm	Conc. µg/mL	Area	AVG	%RSD
289	20	120568	120527.3	0.05489
289		120451		
289		120563		
291	20	125044	122061.3	2.11819
291		120458		
291		120682		

Conclusion

As a result, the current research intends to produce a better, faster, more accurate, and precise analytical approach for estimating Lafutidine. For the estimate of Lafutidine, the suggested analytical approach is simple, inexpensive, quick, sensitive, repeatable, and accurate.

Conflict Of Interests

None declared by the authors.

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