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Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE ESTIMATION OF LAFUTIDINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The objectives of the present research was to develop a simple, precise, economical, accurate, reproducible and sensitive method for the quantitative estimation of lafutidine in bulk and its pharmaceutical dosage forms by Ultra Violet (UV) absorption spectrophotometry.

Methods: The method uses 0.1 N HCl, pH 1.20 as a solvent of choice for the quantitative estimation of lafutidine in bulk and its tablets dosage form by UV absorption spectrophotometry at a wavelength of 290 nm. The method was validated for parameters like linearity, range, precision, Limit of Detection (LOD), Limit of Quantification (LOQ), accuracy, recovery and stability of the analyte.

Results: Lafutidine exhibited absorbance maxima at 290 nm in 0.1 N HCl, pH 1.20 solvent. The developed method was validated as per the ICH validation guidelines. Beer's law was obeyed in range of 0-30 µg/ml with r^2= 0.9997. The LOD and LOQ values of lafutidine were found to be 0.545 µg/ml and 1.654 µg/ml respectively. The mean % recovery for the developed method was found to be in the range of 99.25 to 99.45 % respectively for the marketed dosage forms. The developed method was also found to be robust.

Conclusion: The developed method was found suitable for the routine quantitative analysis of lafutidinein bulk and pharmaceutical dosage form. It was also concluded that developed UV spectrophotometry method was accurate, precise, linear, reproducible, robust and sensitive.

Keywords: Lafutidine, ICH, Validation, Assay, UV spectrophotometry, SGF, Range

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INTRODUCTION

Lafutidine is chemically 2-(furan-2-ylmethylsulfinyl) N-[4-[4-(piperidin-1-ylmethyl) pyridin-2-yl] oxybut-2-en-1-yl] acetamide as shown in fig. 1 [1]. Lafutidine is not official in any pharmacopoeias. It is used as an anti-ulcerating agent as it is the new generation H_2 receptor blocker. It is a H_2 receptor antagonist and is reported to show potent and long lasting antagonisms of histamine H_2 receptor-mediated effect. It is effective agonist the oesophageal lesions induced by acid reflux through inhibition of acid secretions [2]. Oral administration of lafutidine is safe and effective in reducing oral burning symptoms [3]. The earlier studies suggest that therapy with lafutidine is effective and well tolerated in patients with Acid Peptic Disorders (APDs). It is also useful in those patients who were previously not controlled on Proton Pump Inhibitors (PPIs) and first generation H_2 Receptor antagonist. Histamine is a chemical present in mast cells of the body and its release causes the production of acid in the stomach. H_2-blockers inhibit histamine action and therefore reduce gastric secretion or the amount of acid produced. Therefore lafutidine can be used as an empiric therapy to treat APDs. Lafutidine has a biological half-life (~2-3hr) with site-specific absorption in the upper part of the gastrointestinal tract (GIT) and is also stable in gastric pH [4]. Therefore properties of lafutidine make it suitable for the development of controlled or convential drug delivery systems which release the drug in the gastric contents of stomach thus ensuring optimal bioavailability and high therapeutic effects.

Therefore development and validation of a spectroscopic method for estimation in 0.1 N HCl, pH 1.20 (SGF) would be an ideal approach for estimation of lafutidine. The literature survey revealed that various methods of analysis for lafutidine have been reported which include, Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (LC-ESI-MS) [5], High-Performance Liquid Chromatography (HPLC) [6], Liquid Chromatography-Mass Spectrometry (LC-MS) and Ultra Violet UV Spectrophotometric [7] methods.

Some of these methods have enough sensitivity to determine lower concentrations of drug; however, many of these techniques are deficient in simplicity, cost-effectiveness and accessibility. Out of these, UV spectrophotometry or spectroscopy method is featured by its speed, simplicity, accuracy and inexpensiveness. An UV Spectroscopic method has been reported for lafutidine in 0.1 N HCl, pH 1.20 (SFG) solvent and validated according to ICH norms [8].

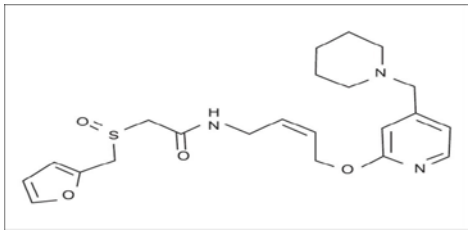

Fig. 1: Structure of lafutidine [1]

MATERIALS AND METHODS

Instrumentation

A double beam UV-Visible spectrophotometer1800, Shimadzu, software version 2.23 using 1 cm matched quartz cell with a slit width of 1 mm was used for all the spectral and absorbance measurements. The scanning speed was kept medium. Digital weighing balance (Shimadzu analytical balance) was used for weighing purpose.

Reagents and chemicals

Pure drug sample of lafutidine with % purity 99.60 was supplied by Pure Chem Pharmaceutical Pvt. Ltd. Gujarat, India. The sample was

used without further purification. Tablet used for analysis was Lafudac manufactured by Unichem Laboratory Ltd. Mumbai and Laciloc manufactured by Cadila Pharmaceuticals Ltd. containing Lafutidine 10 mg per tablet.

All chemicals and reagents were of analytical grade and double distilled water was used throughout the investigation to develop spectral characteristics.

Selection of solvent

0.1 N HCl, pH 1.20 was selected as a solvent to study spectral characteristics of lafutidine. It was prepared according to Indian Pharmacopoeia 2014.

Preparation of standard stock solution

Accurately weighed 10 mg pure drug sample of lafutidine was transferred to 100 ml (100 µg/ml) calibrated volumetric flask, dissolved and made up to the mark with 0.1N HCl, pH 1.20. It was the stock solution of lafutidine (100 µg/ml) in 0.1 N HCl, pH 1.20.

By using the stock solution of 100 µg/ml, subsequently dilution was carried out by withdrawing different aliquots (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml) from standard solution were transferred into a series of 10 ml calibrated volumetric flasks and all were made up to the mark with 0.1 N HCl, pH 1.20 in order to prepare working standard solutions of different concentrations (5-30 µg/ml).

Selection of detection wavelength

A solution of lafutidine in the concentration of 10 µg/ml was scanned in the range of wavelength 200-400 nm [9]. It was observed that the lafutidine showed considerable absorbance at a wavelength of 290 nm. The absorption spectrum was found sharp and maximum at wavelength of 290 nm, therefore, it was selected as the wavelength for detection in 0.1 N HCl, pH 1.20. The study of spectrum revealed that lafutidine shows a well-defined λ_{max} at 290 nm.

Preparation of calibration curve

A solution of lafutidine in 0.1 N HCl, pH 1.20 in different concentrations (5, 10, 15, 20, 25 and 30 µg/ml) was taken and the absorbance of these solutions was measured against solvent 0.1 N HCl, pH 1.20 as blank at a wavelength of 290 nm. A calibration curve was plotted from the absorbance values so obtained [10]. From the calibration curve, it was found that lafutidine obeys Beer's law in concentrations of 5-30 µg/ml. The optical characteristics are summarized in table 1.

Table 1: Calibration curve parameter of lafutidine

S. No.	Concentration (µg/ml)	Absorbance±SD(nm)
1.	0	0
2.	5	0.106±0.002
3.	10	0.203±0.002
4.	15	0.286±0.006
5.	20	0.385±0.001
6.	25	0.482±0.003
7.	30	0.581±0.005

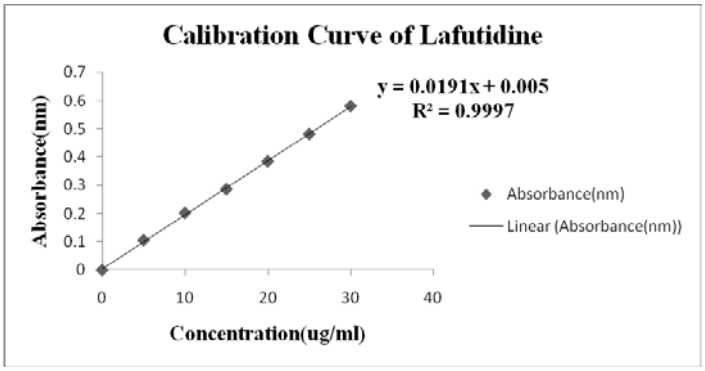

Fig. 2: Calibration curve of lafutidine

Preparation of sample solution

Ten tablets of lafutidine (Lafudac, 10 mg) were weighed accurately and powdered finely. An accurately weighed quantity of tablets powder equivalent to 100 mg of lafutidine was transferred to a 100 ml volumetric flask and diluted with 0.1 N HCl, pH 1.20 and the content was ultrasonicated for 20 min. The volume was made up to the mark with solvent and mixed well. The solutions were further filtered using whatman no.1 filter paper to remove any unwanted particulate matters. The filtered solutions were further appropriately diluted with respective solvent to finally produce sample solution of concentration 10 µg/ml for analysis. The amount of lafutidine present in the sample solution was determined by using the calibration curve of standard drug.

Method validation

The method was validated according to ICH Q2 (R1) guidelines for parameters like linearity, range, precision, LOD, LOQ, accuracy, recovery and stability of the analyte.

Linearity and range

The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. The range was determined to know the interval between the upper and lower concentrations of analyte (pure lafutidine) that had been demonstrated to determine that the analytical method with a suitable level of precision, accuracy and linearity [11].

To establish the linearity and range of the proposed method, various aliquot portions of 0.5-3 ml of lafutidine stock solution (100µg/ml) was separately transferred into 10.0 ml volumetric flask and diluted up to the mark with mobile phase (0.1 N HCl, pH 1.20) to obtain final concentrations of 5-30 µg/ml respectively. The absorbance at each concentration was measured against solvent 0.1 N HCl, pH 1.20 as blank at a wavelength of 290 nm. A calibration curve was constructed by plotting absorbance of lafutidine (nm) on Y-axis and concentration of lafutidine (µg/ml) on X-axis and regression equation was calculated for the drug.

Precision

Precision studies were carried out to ascertain the reproducibility of results for the proposed method and used to find out intra and interday variations in the test method of lafutidine. It was determined by repeatability [12]. Repeatability determined by preparing six replicates of the same concentration of the sample and the absorbance was measured. Intraday precision study was carried out by preparing drug solution of same concentrations (5, 10 and 15 µg/ml) and analysed it at three different times in a day. The same procedure was followed for three other days to determine interday precision. The results were reported as % RSD.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD is the lowest amount of analyte in the study or test sample that can be detected. LOQ is the lowest amount of analyte in the study or test sample that can be quantitatively determined by suitable precision and accuracy [13]. LOD and LOQ were determined by using the following equations designated by ICH guidelines.

LOD–3.3 s/m-----Eq. 1

LOQ–10 s/m-----Eq. 2

Wheres is the standard deviation of the response and m is the slope of the related calibration curve [14].

Accuracy study

Accuracy study was carried out to assure the closeness of the test results obtained by the analytical method to the true value [15]. For study methods, samples were prepared in triplicate at three different concentrations i.e.10 µg/ml,15 µg/ml and 20 µg/ml within the Beer's law limits and the absorbance of each concentration was recorded in triplicate (n=3). The results were reported as SD and % RSD.

Recovery study

Recovery study was carried out to find the accuracy of the proposed method by addition of standard drug solution to a pre-analyzed tablet dosage form of lafutidine sample solution at three different concentration levels (80 %, 100 % and 120%) within the specified linearity and range. The basic concentration level of sample solution selected for spiking of the drug Standard solution was 10 µg/ml of lafutidine for the used method. The % recovery by proposed method was calculated by using the formula as given below.

% Recovery= [(E-T)/P] ×100

E: Total amount of drug estimated (µg/ml) after standard addition

T: Amount of drug found in pre-analyzed tablet dosage form (µg/ml)

P: Amount of pure drug added (µg/ml)

Robustness

Robustness was carried out by analyzing-lafutidine concentration on different days by different analysts. This study was used to predict the effect of various parameters such as different laboratories, different analysts, interday and intraday variations [16-17].

Assay of marketed formulations of lafutidine

The average weight of twenty tablets of a marketed brand of lafutidine was accurately calculated and these tablets were crushed well into a uniform powder. Calculated weight of powdered drug was taken to prepare 100 µg/ml stock solution. Three replicates of the test solution of 10 µg/ml were prepared from dilution of stock solution and sonicated. The amounts of lafutidine were calculated by extrapolating the absorbance from the calibration plot. Results of the analysis are reported in table 7.

RESULTS AND DISCUSSION

Linearity and range

The absorbance of lafutidine at each concentration of 5-30 µg/ml was measured against solvent 0.1 N HCl, pH 1.20 as blank at a wavelength of 290 nm. A calibration curve was constructed by plotting absorbance versus concentrations and a regression equation was calculated for drug. The equation of calibration curve for lafutidine obtained was y = 0.005+0.019x, Good linearity was observed over the concentration range evaluated (5-30 µg/ml) with regression coefficient (r^2)=0.9997 are shown in table 1 and fig. 3. The calibration curve was found to be linear for the proposed spectrophotometric method in the aforementioned concentrations (5-30 µg/ml). The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over a wide range.

Table 2: Various parameters of developed methods of lafutidine

Parameter	Result
Absorption Maxima	290 nm
Beers law Range	0-30 µg/ml
Correlation Coefficient	0.9994
Regression Coefficient	0.9997
Regression Equation	Y= 0.005+0.019X
Slope	0.0191
Intercept	0.0050
Standard deviation (SD)	0.00316
LOD, µg/ml	0.545
LOQ, µg/ml	1.654

Precision

In the intraday study, the concentration of drug was calculated on the same day at an interval of two hour. In the interday study, the concentration of drug contents was calculated on three different days within laboratory variation. In both intraday and interday precision, study was expressed as relative standard deviations (RSD) of a set of results. The precision of the method (% RSD) of lafutidine for intraday and interday was found to be less than 1% in each concentration showing good repeatability. The values of % RSD for both intraday and interday precision are shown in table 3.

Table 3: Determination of intraday and interday precision for three different concentrations of lafutidine

S. No.	Normal concentration (µg/ml)	Intraday precision (n=3)*		Interday precision (n=3)*	
		Concentration measured (µg/ml) [mean±SD]	% RSD	Concentration measured (µg/ml) [mean±SD]	% RSD
1	5	4.97±0.015	0.31	4.95±0.30	0.62
2	10	9.96±0.025	0.25	9.97±0.025	0.25
3	15	15.01±0.025	0.17	14.98±0.055	0.37

*All values are expressed as mean±SD, n=3

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and limit of quantification of lafutidine by proposed method was determined using standard deviation method with calibration standards. The LCD and LOQ of the proposed method were found to be 0.545 µg/ml and 1.654 µg/ml respectively indicating that the method developed is sensitive and without the interference of the excipients.

Accuracy study

The accuracy of the method was determined and results of the study showed a low value of % RSD indicating an accurate method (table 4) as well as non-interference with the excipients of the formulation.

Recovery study

To perform the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. All value come under 100±1 % (table 5) that indicate method is accurate.

Robustness

Robustness study showed the non-significant difference in the amount of drug recovery (% assay) in different laboratory, analyst and variations condition. Robustness study suggested that developed method was independent in different parameters of environmental conditions.

Table 4: Accuracy data for three different concentrations of lafutidine for the developed method

S. No.	Concentration (µg/ml)	Absorbance measured (mean±SD) (n=3)*	% RSD
1	10	0.204±0.0015	0.75
2	15	0.288±0.0020	0.72
3	20	0.387±0.0020	0.54

*All values are expressed as mean±SD, n=3

Table 5: Result of recovery study by percentage recovery method

Marketed dosage form	Content of drug (mg)	Level of addition (%)	% recovery (n=3)*±% RSD	% mean recovery±% RSD
	10	80	99.62±0.47	
Lafudac 10 mg	10	100	99.51±0.25	99.45±0.20
tablet	10	120	99.23±0.54	
Laciloc 10 mg	10	80	99.44±0.75	99.25±0.17
tablet	10	100	99.18±0.58	
	10	120	99.13±0.82	

*All values are expressed as mean, n=3

Table 6: Data for robustness study for the developed method

S. No.	Variable parameter	Assay result (%) (n=3)*
1	Analyst 1	99.7
	Analyst 2	99.7
	Analyst 3	99.8
2	Day 1	99.7
	Day 2	99.6
	Day 3	99.6

*All values are expressed as mean, n=3

Table 7: Assay of marketed dosage form of lafutidine

Marketed dosage form	Label claim (mg)	Amount found (mg) (n=3)*	% Purity ±% RSD
Lafudac 10 mg Tablet	10	9.94	99.40±0.45
Laciloc 10 mg Tablet	10	9.92	99.20±0.68

*All values are expressed as mean, n=3

Assay of marketed formulations of lafutidine

The assay results (% purity) of 20 tablets of different marketed brands (Lafudac and Laciloc) were found to be 99.40 %±0.45 and 99.20 %±0.68 respectively. The estimated content (% purity) was in good agreement with the label claims.

CONCLUSION

A UV-Spectrophotometric method was developed and validated as per the ICH guidelines for lafutidine determination in bulk and pharmaceutical dosage form. The solvent (0.1 N HCl, pH 1.20) used for this study was inexpensive, appropriate and simple to prepare. The developed analytical method was found to be simple, sensitive, rapid, economical, linear, reproducible, robust and applicable over a wide concentration range with high interday, intraday precision and accuracy. The results of the validated parameters were found to be satisfactory and can also be applied for the quality control tool in the estimation of lafutidine in pharmaceutical dosage forms. The method was found suitable to determine the concentration of lafutidine as API as well as in dosage form analysis precisely and accurately. The sample recovery from the formulation by using this method was very applicable in respect to its label claim.

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CONFLICT OF INTERESTS

There are no conflicts of interest

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