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RP-HPLC DETERMINATION OF LAFUTIDINE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple, precise and accurate RP – HPLC method have been developed and validated for the determination of Lafutidine in bulk and pharmaceutical dosage form. The Chromatography was performed by using Enable ODS reverse phase (250 mm x 4.6 mm, 5 µm particle size) C_{18} column with mobile phase comprising of Acetonitrile: (1% v/v) Glacial acetic acid in the ratio of 70**:**30 v/v and was delivered at a flow rate of 1 ml/min, with UV detection at a wave length of 265 nm. The retention time of Lafutidine was found to be 2.921 min. The developed method showed linear response (r^2=0.9989) in the range of 10-80 µg/ml. The proposed method was validated as per ICH guideline and can be applied for estimation of Lafutidine in pharmaceutical dosage forms in routine analysis.

Key words: Lafutidine, RP - HPLC, Validation.

Introduction

Lafutidine[1] (N-(4-(4-piperidinylmethyl) pyridyl-2-oxy) butenyl-2-(furfurylsulfinyl) acetamide, Fig.1) is a second generation H_2 receptor antagonist with anti-ulcer and hepatoprotective activity. It is indicated for the treatment of peptic ulcer and gastro-oesophageal reflux disease (GORD)[2].

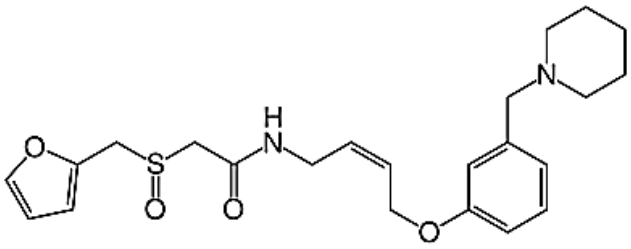

Fig. 1: Structure of Lafutidine

Literature survey reveals that a few methods were reported for the determination of Lafutidine in pharmaceutical formulation which includes - UV Spectroscopy[3-5], LC-MS/MS [6-8], HPLC[9], HPTLC[10]. Other RP-HPLC methods for the determination of Lafutidine include - Determination of Lafutidine in serum with reverse high performance liquid chromatography[11], Determination of related substance in lafutidine by RP-HPLC[12], Stability indicating RP – HPLC method for the simultaneous estimation of Domperidone and Lafutidine in bulk and pharmaceutical dosage form[13], RP-HPLC method for the simultaneous estimation of Lafutidine and Rabeprazole Sodium in combined dosage form[14]. The main objective of the present study was to develop simple, accurate and precise RP-HPLC method for estimation of Lafutidine in bulk and pharmaceutical formulations.

Materials and Methods

Chemicals and reagents

Analytically pure sample of Lafutidine with purities greater than 99 % was obtained as gift sample from Dr.Reddy's Laboratories Hyderabad, India and Tablet formulation [LAFAXID-10] was procured from APOLLO Pharmacy, Visakapatnam, India with labelled amount 10 mg of Lautidine. Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade) were obtained from Merck India. Glacial acetic acid used was of analytical grade of S.D FINE made. A 0.2 µm Nylon membrane filters were obtained from Pall Corporation Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatography comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "LC solutions" software. An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AY 220), p^H meter (Elico 201) a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Elico SL 210, software-Spectral Treats) were used in this study.

Method

Selection of wavelength: Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Lafutidine. Suitable wavelength selected was 265 nm.

Chromatographic conditions

The developed method uses a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5 µ), mobile phase consisting of Acetonitrile and 0.1% v/v Glacial acetic acid in the proportion of 70:30 v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 265 nm.

Preparation of 0.1% v/v Glacial acetic acid

Accurately measured 0.1 ml Glacial acetic acid was transferred into a 100 ml of volumetric flask and volume was made up to the mark with HPLC grade water. The solution was sonicated for 15 min and filtered through 0.2 µm membrane filter.

Preparation of Mobile Phase

The mobile phase was prepared by mixing Acetonitrile and 0.1% v/v Glacial acetic acid in the ratio of 70:30 v/v . It was sonicated for 10 minutes and filtered through 0.2 µm membrane filter.

Preparation of working standard solution

10 mg of Lafutidine was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 2 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as working standard solution (100 µg/ml).

Preparation of stock and working sample solution

Twenty Tablets were weighed separately and the average weight was determined. The tablet content equivalent to 100 mg of Lafutidine was transferred to a 100 ml volumetric flask and dissolved in little portion of mobile phase then volume was made up to the mark with mobile phase. The resulting solution was sonicated for 3 minutes, followed by filtration through 0.2 µ nylon membrane filter to get sample stock solution of 1mg/ml. 1 ml of the above stock solution was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of working standard of 100 µg/ml.

Results and Discussion

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the

elution of Lafutidine at 2.921 min. Fig. 2 represents standard solution (100µg/ml). The total run time is 5 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*) and peak Asymmetric factor were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

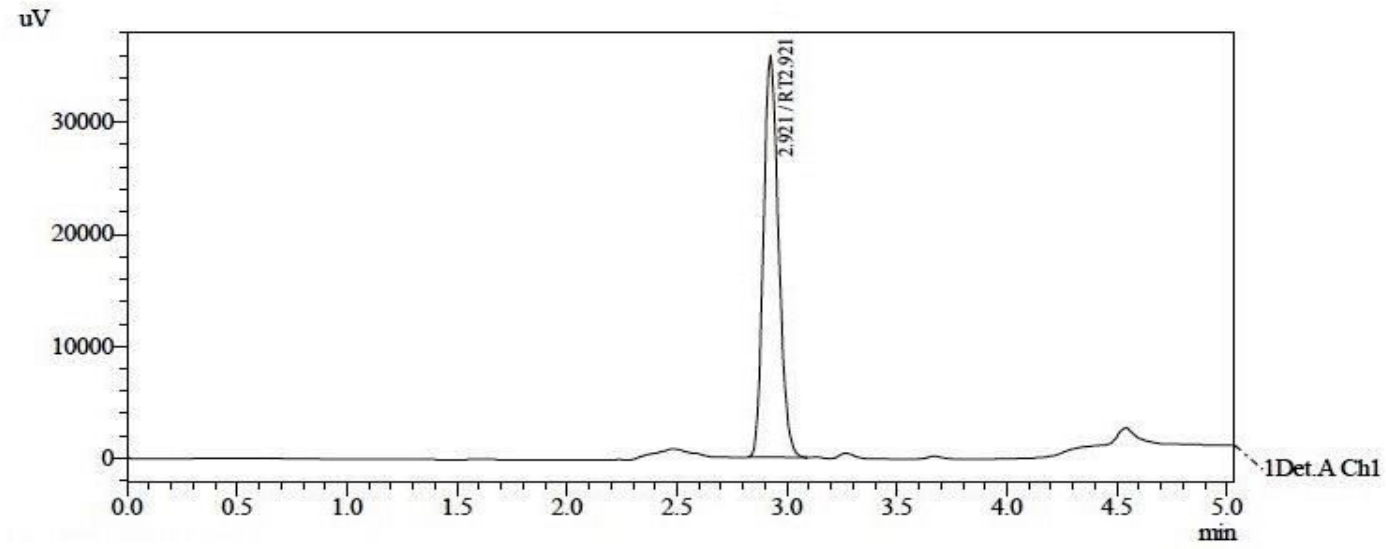

Fig.2: Chromatogram of Standard Lafutidine solution.

Table-1: Results from system suitability studies.

Property	Values ± SD*	%RSD	Required Limits
Retention time (min)	2.92±0.0066	0.26	RSD<1%
Theoritical plates (N)	5704±14.88	0.56	N>2000
Tailing factor (T)	1.05±0.0223	1.421	T<2

*Average of six determinations

In order to test the applicability of the developed method to a commercial formulation, "LAFAXID-10" was chromatographed at working concentration (100µg/ml). The sample peak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

Method validation [15]

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of Lafutidine from excipients. There is no interference of excipient peak on the peak of lafutidine indicating the high specificity of method.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies.

Intra-day Precision

In the intraday studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. %RSD was calculated and is presented in Table 2.

Table-2: Intra-day precision results for Lafutidine.

S.No	Concentration (µg/ml)	Retention time (min)	Peak Area
1	40	2.921	486621
2	40	2.911	486654
3	40	2.919	484531
4	40	2.890	484532
5	40	2.918	487965
6	40	2.919	484573
AVG		2.913	485812
SD		0.0117	1470.6
%RSD		0.40	0.302

Inter-day Precision

In the inter-day variation studies, six injections of standard solution were injected at different days. % RSD was calculated and is presented in Table 3.

Table-3: Inter-day precision results for Lafutidine.

S.No	Concentration (µg/ml)	Retention time (min)	Peak Area
1	40	2.920	483421
2	40	2.916	484563
3	40	2.922	485432
4	40	2.898	486543
5	40	2.912	487951
6	40	2.922	486543
AVG		2.915	485742.1
SD		0.0091	1614.6
%RSD		0.312	0.33

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120 %). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 4**.** The accepted limits of recovery are 98 % - 102 % and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table-4: Results from recovery studies.

Sample	Area	Sample amount (µg/ml)	Standard added (µg/ml)	Standard recovered* (µg/ml)	% Recovery ± SD*	%RSD
80%	389304	50	40	39.93	99.83±0.03	0.030
100%	486623	50	50	49.81	99.63±0.043	0.0431
120%	583964	50	60	59.86	99.77±0.032	0.032

*Average of three determinations

Linearity

Standard solutions of Lafutidine at different concentrations were prepared. Calibration curve was constructed by plotting the concentration of drug versus corresponding peak area. The results show an excellent correlation between peak area and concentration of drug within the concentration range (10-80 µg/ml) for the drug and the results are given in Tables 5 & 6. The correlation coefficient of Lafutidine is greater than 0.99, which meet the method validation acceptance criteria and hence the method is said to be linear. The linearity plot was shown in Fig.3.

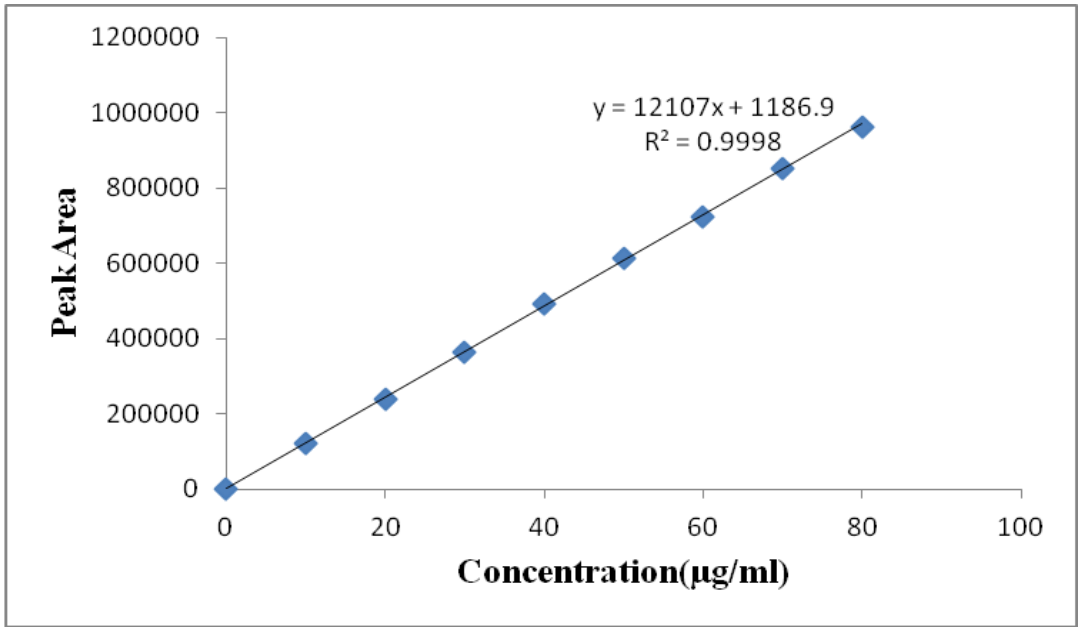

Fig.3: Linearity plot of Lafutidine.

Table-5: Calibration data of Lafutidine.

S.No	Concentration (µg/ml)	Peak Area
1	10	120854
2	20	241354
3	30	362685
4	40	491235
5	50	612386
6	60	725432
7	70	851982
8	80	963215

Table-6: Characteristic parameters of Lafutidine.

Parameters	RP-HPLC
Calibration range (µg/ml)	20-80
Detection Wavelength(nm)	265
Mobile phase (Acetonitrile :Methanol) (V/V)	70:30
Regression equation (Y)	12107x+1186.9
Retention Time(min)	2.921
Slope (b)	12107
Intercept (a)	1186.9
Correlation coefficient (r^2)	0.999
LOD (µg/ml)	0.389
LOQ (µg/ml)	1.16

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in wave length and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results were shown in Table 7.

Table-7: Robustness studies of Lafutidine.

S.No	Condition	Modification	Mean Peak area ± SD*	Mean Rt ± SD*	Mean %RSD (for AREA)
1	Flow rate (ml/min)	0.9	485321±1654	4.213	0.340
		1.1	498754±1876	3.898	0.376
2	Wavelength (nm)	263	472876±1521	4.243	0.321
		268	457642±1562	4.153	0.341

*Average of three determinations

Ruggedness

It was checked by determining precision on same instrument, but by a different analyst. Results of reproducibility are shown in Table 8.

Table-8: Ruggedness studies of Lafutidine.

S.No	Injection Number	Analyst - 1			Analyst-2		
		Area	Retention time (min)	Theoretical plates (N)	Area	Retention time (min)	Theoritical plates (N)
1	1	485324	4.153	5613	488761	4.121	5665
2	2	487854	4.167	5551	486532	4.138	5442
	AVG	486583	4.16	5582	487646	4.129	5553
	SD	1788.9	0.0098	69.29	1576.1	0.012	86.97
	%RSD	0.38	0.23	0.12	0.33	0.29	1.5

Sensitivity

The sensitivity of measurement of Lafutidine by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 0.389µg/ml and 1.16µg/ml.

Estimation of Lafutidine in Pharmaceutical Dosage Form

The proposed method was successfully applied for the estmation of lafutidine in Tablets. The assay results was shown in Table 9.

Table-9: Assay studies of Lafutidine.

Sample	Label Claim (mg)	Standard Area*	Sample Area*	Amount found (mg)	(%) Recovery± SD
LAFAXID-10	10	487035	486811	9.95	99.5± 0.03

*Average of three determinations

Conclusion

A high performance liquid chromatography method for the quantitative estimation of Lafutidine in bulk and tablet dosage form has been developed. The method was validated and found to be applicable for the routine analysis of Lafutidine in tablet dosage forms without interference from the excipients. Statistical results and low % RSD values

indicate that the method is precise, accurate, robust, and specific. Considering already proposed methods in literature, advantages of this new proposed method are rapid analysis, economic mobile phase, user friendly and convenient approach. All these key features proposed that this method can be considered as advantageous over other methods.

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