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A stability-indicating high performance liquid chromatographic analytical method for the determination of Lafutidine in Tablet

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ABSTRACT

The present study describes the development and validation of a stability indicating reverse phase HPLC (RP-HPLC) method for the analysis of lafutidine, an H_2 receptor antagonist drug, in tablet dosage forms. The proposed RP-HPLC method utilizes Shisheido C18, 250mm x 4.6mm, 5µm column (at ambient temperature), isocratic run (using methanol and acetonitrile as mobile phase), at a flow rate of 1.0 ml/minute, and UV detection at 273nm for analysis of lafutidine. The reported method is linear over the range of 75-200µg/ml. The precision, ruggedness and robustness values were also within the prescribed limits. Lafutidine was exposed to acidic, alkaline, oxidative, thermal and photolytic stress conditions- and the stressed samples were analyzed by the proposed method. Chromatographic peak purity results indicated the absence of co-eluting peaks with the drug peak, which demonstrated the specificity of assay method for estimation of lafutidine in presence of degradation products. The proposed method can be used for routine analysis stability testing of lafutidine in quality control laboratories.

Key words: Reverse-phase-HPLC; Lafutidine; Stability indicating assay; Forced degradation

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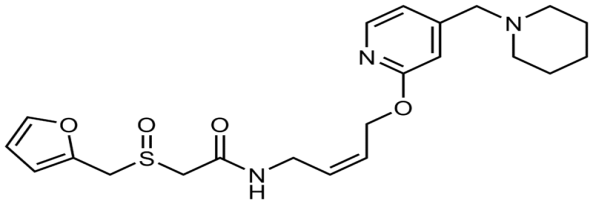
INTRODUCTION

Lafutidine is a H_2 receptor antagonist drug [1]. The chemical name for lafutidine is (±)-2-(Furfurylsulfinyl)-*N*-[4-[4-(piperidinomethyl)-2-pyridyl] oxy-(*Z*)-2-butenyl] acetamide]. Lafutidine is a Yellowish White Crystalline Powder with the molecular formula of $C_{22}H_{29}N_3O_4S$ and a molecular weight of 431.56**.** It is freely soluble in DMF, Glacial acetic acid, soluble in methanol, sparingly soluble in dehydrated ethanol, very slightly soluble in ether, practically insoluble in water. [2-3]. Fig.1, shows the structural formulae of lafutidine. To date, published analytical methods are all estimation of lafutidine with reversed-phase high performance liquid chromatography (RP-HPLC) but the present study is the first time report on stability indicating assay of lafutidine in presence of degradation products by HPLC. In this method isocratic elution method is selected for the analysis of lafutidine because it gave better base line separation and peak shape, which is suitable for the routine analysis of lafutidine. In view of above in the present study we hereby report the development and validation of a stability indicating isocratic reverse-phase HPLC (RP-HPLC) method for analysis of lafutidine in presence of degradation products as per ICH guidelines [4-5].

Fig.I. Structural formulae for lafutidine



MATERIALS AND METHODS

Chemical and Reagents Lafutidine standard and tablet were provided by Unichem Healthcare Ltd, Mumbai, India. Acetonitrile and hydrogen peroxide were from Qualigens, Mumbai, sodium hydroxide and hydrochloric acid were from Merck (Darmstadt, Germany). All chemicals were at least of analytical grade and used as received. Purified HPLC grade water was obtained by reverse osmosis and filtration through a TKA smart 2 pure (Germany) and was used to prepare all solutions.

HPLC Instrumentation and Conditions The HPLC system— Cyber lab LC 100 plus (USA) separation module having maximum pressure of 5000 psi, detector—LC-UV 100 Plus, Photo diode array detector. To develop a precise, linear, specific and suitable stability indicating RP-HPLC method for analysis of lafutidine, different chromatographic conditions were applied and the results are presented in Table 1 and the following optimized conditions were applied for final analysis. The chromatographic separation was performed using shiseido column, 250mm **X** 4.6 mm i.d. 5µ particle size column (at ambient temperature), isocratic run, at a flow rate of 1.0 ml/minute, and UV detection at 273 nm for analysis of lafutidine. The mobile phase consisted of methanol – acetonitrile (90:10) filtered through 0.45µm nylon filter and degassed in ultrasonic bath prior to use. Wavelength was selected by scanning standard solution of drug over 200 to 400 nm using double beam UV–visible spectrophotometer (model Analytical T20 plus U.K). Measurements made with lafutidine tablet and ultraviolet (UV) detection at 273nm, showed reasonably good response.

Preparation of solution:

Standard solution

A solution of Lafutidine was prepared by accurately weighed and transferred about 10mg of Lafutidine reference/working standard in to a clean 50mL volumetric flask, than 25mL of mobile phase was added and sonicated for 5min to dissolve the Lafutidine , volume was made up to the mark with mobile phase this gave a stock solution of concentration 200µg/mL. From this stock solution, 5mL of solution was pipette out and transferred to a 10mL clean volumetric flask and the volume was made up to the mark with mobile phase this result in standard solution of concentration 100µg/mL.

Sample solution

A sample solution of Lafutidine was prepared by accurately weighed and transferred powdered tablet equivalent to 10mg drug in to a clean 50mL volumetric flask, 25mL of mobile phase was added and sonicated for 5min to dissolve the Lafutidine , volume was made up to the mark with mobile phase, this gave a stock solution of concentration 200µg/mL. From the stock solution 5mL solution was pipette out and transferred to a clean volumetric flask and volume was made up to the mark with mobile phase this result in sample solution of concentration 100µg/mL. The sample solution (100µg/mL) was filtered through 0.45µm nylon filter and degassed in ultrasonic bath prior to use.

Blank solution

Methanol: Acetonitrile (90:10) was used as a blank solution (Diluent).

Placebo solution

Methanol: Acetonitrile (90:10) was used as a placebo Solution.

Optimization of Chromatographic Conditions The chromatographic conditions were optimized by different means. [Using different column, different buffer and different mode of HPLC run].

Specificity (Forced Degradation Studies) Lafutidine was allowed to hydrolyze in base (0.1N NaOH), acid (0.1N HCl) and hydrogen peroxide (10% v/v). Lafutidine was also studied for its thermal degradation at 80°C for 2 days and photolytic degradation for 10 days, exposed to white fluorescent light (1.2 million lux) near UV fluorescent light (200 w/m^2). Lafutidine powdered tablet was accurately weighted 10mg transferred in clean 100mL volumetric flask

with 20 ml of base (NaOH), acid (HCl) or hydrogen peroxide 10% v/v (H_2O_2) with 20 ml of water and kept at room temperature to study the degradation, 5 ml of sample was taken at various time interval (Time schedule is given in Table-1) and it was neutralized with 0.1 N HCl or 0.1 NaOH solution and diluted with water to get the final concentration of 100µg/mL of lafutidine. Similarly placebo solution was prepared. Sample and placebo solutions were analyzed as per methodology, calculated the percentage degradation. The results of stability studies are presented in Table 2.

Table I. Sampling plan

Time point	Base stress	Acid stress	Peroxide stress	Heat stress	Light stress
Initial	√	√	√	√	√
4 Hrs.	√	√	√		
8 Hrs.	√	√	√		
24 Hrs.	√	√	√	√	
2 days				√	
5 days					√
10 days					√

Table II. Results of Force Degradation Studies of Carboplatin

Stress condition/duration/solution	% degradation
Alkaline degradation (0.1 N NaOH, 4 h)	20.2
Alkaline degradation (0.1 N NaOH, 8 h)	20.2
Alkaline degradation (0.1 N NaOH, 24 h)	36.0
Oxidative degradation (10% H_2O_2, Initial)	24.4
Oxidative degradation (10% H_2O_2, 1 h)	33.7
Oxidative degradation (2% H_2O_2, Initial)	17.4
Oxidative degradation (2% H_2O_2, 1 h)	21.6
Oxidative degradation (2% H_2O_2, 4 h)	24.6
Acidic degradation (0.1 N HCl, 4 h)	21.3
Acidic degradation (0.1 N HCl, 8 h)	29.0
Acidic degradation (0.1 N HCl, 24 h)	32.0
Thermal degradation (liquid sample, 24 h)	2.1
Thermal degradation (liquid sample, 48 h)	2.3
Photolytic degradation (UV fluorescent light 200 w/m^2, 5 days)	3.1
Photolytic degradation (UV fluorescent light 200 w/m^2, 10 days)	5.0

Validation:

Linearity was determined by injecting different concentration of sample solutions (75-200µg/ml, Fig. 3). For system precision, standard solution (100µg/ml) was injected in six replicates to check % RSD (relative standard deviation) and for method precision six samples were prepared and each of these were injected in duplicate. Mean of all of these values gives rise to assay value. To establish the within-day (intra-assay) and between-day (inter-assay) accuracy and precision of the method, lafutidine was assayed on one day and three separate days. Intra-assay and inter-assay were calculated and the data are presented in Tables 3 and 4 respectively. Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate (10%), organic content in mobile phase (2%), wavelength of detection ((±2nm) different batch of column and column oven temperature (±2°C). Robustness of the developed method was indicated by the overall % RSD between the data at each variable condition.

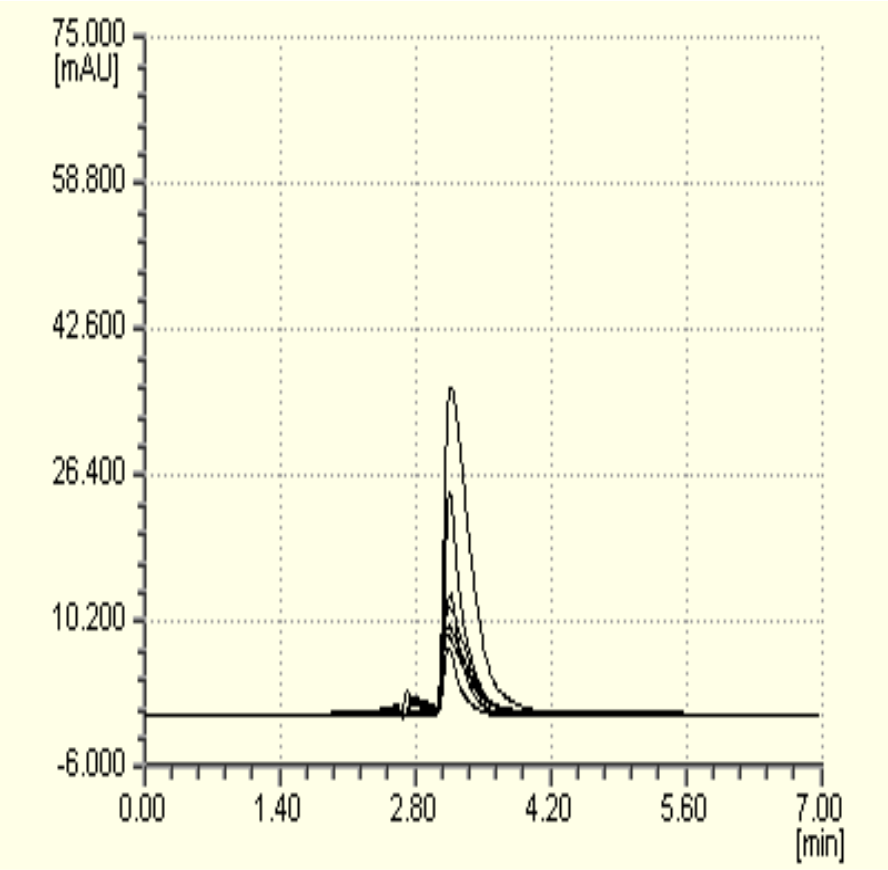
Table III. Intra-assay Precision Data of Proposed RP-HPLC Method (Method Ruggedness)

	Mean (% w/w)	S.D. (n=6)	% RSD
Assay-1	100.3	0.252	0.25
Assay-2	100.2	0.115	0.12
Intra assay	100.2	0.183	0.18

Table IV. Inter-assay Precision Data of Proposed RP-HPLC Method

	Mean (% w/w)	S.D. (n=6)	% RSD
Analyst-1	100.9	0.022	0.22
Analyst-2	100.05	0.259	0.25
Inter assay	100.5	0.052	0.51

Fig. III. Overlain chromatogram of lafutidine



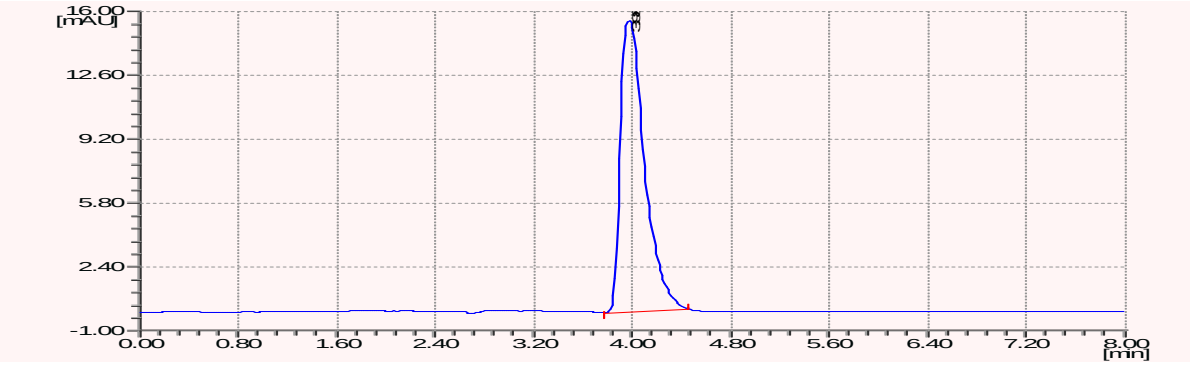
Stability in Analytical Solution The stability of the standard and sample solution were demonstrated by analyzing the standard and sample solutions at regular time intervals (i.e. 4 hrs.) till 24 hrs, keeping the solution at room temperature and refrigerator (2 to 8°C). The area counts of the lafutidine peak in the standard and sample solutions were calculated, the cumulative RSD for area counts were calculated.

RESULTS AND DISCUSSION

Chromatographic Conditions To develop a precise, linear, specific and suitable stability indicating RP-HPLC method for analysis of lafutidine, different chromatographic conditions were applied and the results observed are presented in Table- 1. Isocratic elution is simple, requires only one pump and flat baseline separation for easy and reproducible results. In case of RP-HPLC various columns are available, but here Shiseido, 250mm**X**4.6 mm i.d. 5µ column was preferred because using this column peak shape, resolution and absorbance were good and acetonitrile was selected as mobile phase, because of its favorable UV transmittance. Among the different mobile phases composition employed the mobile phase consisted of methanol - Acetonitrile (90:10) was found to be suitable for the analysis of lafutidine. Further, a flow rate of 1.0 ml/min, an injection volume of 20µl and UV detection at 273nm was found to be best for analysis.

Forced Degradation Studies:- In the present study even though the conditions used for forced degradation are in the range of producing 20-80% target degradation, degradation of lafutidine could not be achieved even after prolonged duration. During the study it was observed that upon treatment of lafutidine with base (0.1 N NaOH), acid (0.1 N HCl) and hydrogen peroxide 10% v/v (H_2O_2) the degradation was observed. In case of heat after 2 days was found 2.38% degradation, and in case of light after 10 days 5.0% degradation was found. Table-3 shows the extent of degradation of carboplatin under various stress conditions. The peak of carboplatin is unaffected (fig. 2), which indicates that these degraded peaks are due to the presence of impurities and the lafutidine is stable under the applied stress conditions like heat, light, acid, alkaline hydrolysis and oxidative degradation states.

Fig.II. Chromatogram of lafutidine



Linearity The calibration curve showed good linearity in the range of 75-200µg/ml, for lafutidine with correlation co-efficient (r^2) of 0.999 (Fig. 3). A typical calibration curve has the regression equation of y = 0.005x - 0.041 for lafutidine.

Precision The results of system precision (% RSD 0.54) and method precision (% RSD 0.61) are found within the prescribed limit of ICH guidelines (% RSD <1%, and % RSD <2% respectively in case of system precision and method precision).

Intra-assay and Inter-assay The intra- and inter-day variation of the method was carried out and the high values of mean assay and low values of standard deviation and % RSD (% RSD <2%) within a day and day to day variations for lafutidine revealed that the proposed method is precise (Tables 3-4).

Accuracy, as Recovery

Accuracy of the method was carried out by recovery studies. A preanalysed sample of lafutidine was spiked with standard drug at three different levels (80%, 100% and 120%). Each solution were prepared in triplicate and analyzed in triplicate after suitable dilution. The recovery data (% RSD) obtained at each level was < 2%. The average recovery yield at three different levels of 80%, 100% and 120% were found to be 98.75, 98.5 and 98.93 respectively. Since the results obtained were within the acceptable range 98.0 to 102.0%, the method was deemed to be accurate. The accuracy results are summarized in Table 5.

Table V. Accuracy data

Interpolated concentration [mean±SD (*n*=3)]		RSD (%)
Carboplatin concentration (mg/ml)		
0.8	0.803 ± 0.114	0.13
1.0	0.997 ± 0.253	0.26
1.2	1.193 ± 0.207	0.20

Method Robustness Influence of small changes in chromatographic conditions such as change in flow rate ((±10%)), organic content in mobile phase (±2%), wavelength of detection (±2nm), different batch of column and column oven temperature (±2°C) studied to determine the robustness of the method are also in favor (% RSD <2%) of the developed RP-HPLC method for the analysis of lafutidine.

Specificity and Stability in Analytical Solution The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the lafutidine standard and sample solutions were stable at regular time intervals (i.e. 4 hrs.) till 24 hrs, keeping the solution at room temperature and refrigerator (2 to 8°C). Lafutidine was stable in solution form upto 24 h at 25 °C. The results of linearity, precision, inter and intra-day assays, method robustness and specificity and stability in analytical solution established the validation of the developed RP-HPLC assay for the analysis of lafutidine.

CONCLUSION

In conclusion, a sensitive and selective stability indicating RP-HPLC method has been developed and validated for the analysis of lafutidine tablet. Based on peak purity results, obtained from the analysis of force degraded samples using described method, it can be concluded that the absence of coeluting peak along with the peak of lafutidine indicated that the developed method is specific for the estimation of lafutidine in presence of degradation products. Further the proposed RP-HPLC method has good sensitivity, precision, and reproducibility. Even though no attempt

was made to identify the degraded products, proposed method can be used as a stability indicating method for assay of lafutidine tablet.

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