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Analytical method development and validation for the determination of lafutidine using reverse phase HPLC method in bulk and tablet dosage form

R. Vani[1]*, B. Vijaya Kumar[2] and G. Krishna Mohan[3]

[1]*JNTU-K, Kakinada & Deccan School of Pharmacy, Hyderabad*
[2]*Jangaon Institute of Pharmaceutical Sciences, Jangaon*
[3]*Center for Pharmaceutical Sciences, JNTU-H Hyderabad*

ABSTRACT

A new simple, accurate, precise and reproducible RP-HPLC method has been developed for the estimation of Lafutidine in bulk and pharmaceutical dosage form using C18 column (zodiac, 250 x 4.6 mm, 5 µm) in isocratic mode. The mobile phase consisted of A mixture of 30 volumes of 0.1MPhosphate buffer (KH_2PO_4) pH 4.0 and 70 volumes of Methanol is used v/v. The detection was carried out at 299 nm. The method was linear over the concentration range 60-140 µg/ml. The recovery of Lafutidine was found to be 100.29% . The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing Lafutidine in tablet dosage form.

Keywords: Lafutidine, reverse phase HPLC, validation.

INTRODUCTION

Chemically it is (±)-2-(furfurylsulfinyl)-N-[4-[4-(piperidinomethyl) -2-pyridyl]oxy-(Z)-2-butenyl]acetamide .It is a Second generation histamine H2-receptor antagonist. It is used in the treatment of gastric ulcers, duodenal ulcers and gastric mucosal lesions associated with acute gastritis and chronic gastritis. Lafutidine works by interfering with the histamine molecule's ability to bind to parietal cells in the wall of the stomach. These cells release acid into the stomach when they bind with histamine. Preventing this binding decreases the overall amount of acid in a patient's stomach.

Brand name- LAFAXID-10

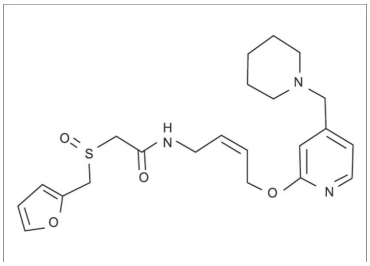

Fig 1: Chemical Structure of Lafutidine

According to literature survey few spectrophotometric, HPLC and methods have been reported for the determination of LAFin single and in combination with other drugs.. However very few HPLC methods were reported for the estimation of LAFin its tablet dosage form. The aim of present work was to develop and validate as per ICH guidelines , a sensitive HPLC method that can be applied for estimation of Lafutidine.

MATERIALS AND METHODS

Materials

Lafutidine was received gratis from Hetero drugs, Hyderabad and was used as received. HPLC grade Methanol was purchased from SD Fine Chem Pvt. Ltd. (Mumbai, Maharashtra). Ultra-pure water was obtained from ELGA (Bucks, UK) water purification unit. All other chemicals were of analytical reagent grade.

Chromatographic conditions

The HPLC system (Agilent 1220 LC) consisted of quaternary gradient system (600 Controller), in-line degasser (Agilent, model AF), VWD detector (Agilent, 2998 model) and manual sampler (Agilent, model 717 plus). Data was processed using EZchrome software (Agilent, Milford, MA, USA).

Isocratic elution of the mobile phase 0.1 M Dipotassium Phosphate buffer (pH 4) and methanol in the ratio of 70:30 v/v with the flow rate of 1 ml/min. Separation was performed on a zodiac C_{18} (250 x 4.6 mm i.d, 5 µ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the Ezchrome software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 µm membrane filter and degassed by sonication before use. Mobile phase was used as diluents.

The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm. The run time was set at 8 min and a column temperature was maintained at 35°C. The volume of injection was 10 µl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluent was detected at 299 nm . The developed method was validated in terms of specificiy, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of Lafutidine and as per ICH guidelines.

Preparation of standard solutions

Lafutidine was weighed (100 mg each) and transferred to 100 ml volumetric flask and dissolved in 50 ml of methanol and make up the volume up to the mark with mobile phase. Working standards of the drugs were prepared from this solution.

Preparation of sample solution:

Twenty tablets (Laciloc Make Cadila) were weighed. An accurately weighed amount of the finely powdered tablets equivalent to 10 mg of Lafutidine and was made up to 100 mL with mobile phase. The solution was filtered followed by serial dilution to the required concentrations for each experiment.

RESULTS AND DISCUSSION

Method Development:

Number of mobile phase and their different proportionswere tried and finally was selected as 0.1 M Dipotassium Phosphate buffer (pH 4) and methanol in the ratio of 30:70 v/v appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The results of system suitability parameters were shown in table 2. The chromatogram of working standard solution is shown in Fig 2. The summaries of Chromatographic conditions were given in table 1.

Method Validation:

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantitiesof pure standards at three different levels in 80%, 100%and 120% to the pre analysed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate. The results were tabulated in Table 3.

Table 1: Summary of Chromatographic conditions

S. No	Parameter	Description/Value
1.	Stationary Phase	ZodiacC18 (250X4.6X5)
2	Mobile Phase	0.1 M Dipotassium Phosphate buffer (pH 4) and methanol in the ratio of 70:30 v/v
3	Flow rate	1 ml/min
4	Detection Wavelength	Lafutidine – 299nm
5	Detector	VWD
6	Rt's	Lafutidine5.489min
7	Injection volume	10 µl
8	Column Temperature	35 °C
9	Run time	8 mins
10	Diluent	Mobile Phase

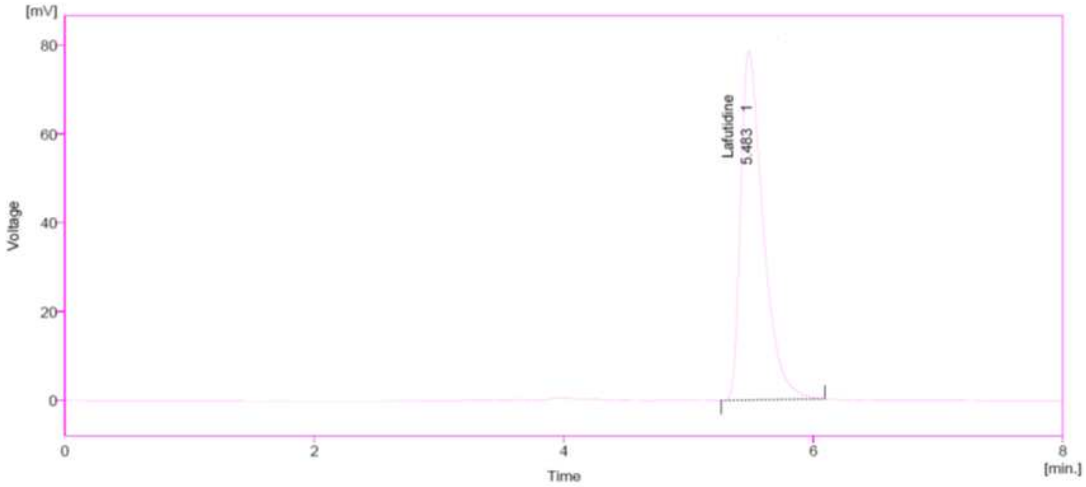

Fig.2 Typical Chromatogram of Lafutidine

Table 2: System suitability parameters

S. No	Parameter	Result
1	Retention Time	Lafutidine5.489min
2	Tailing	1.876
3	Theoretical Plates (n)	4489

Table 3: Results of Accuracy

S. No	% Concentration (at specific level)	Lafutidine		
		Amount added (µg)	Amount found (µg)	Mean % Recovery
1	80	(80+20)	101.93	101.93*
2	100	(100+20)	120.60	100.50*
3	120	(120+20)	142.13	101.52*

**Mean % Recovery of 3 replicates*

Precision

The intraday and interday precision of the proposed method was determined by analyzing the standard solution of lafutidine at concentration 100 µg/mL , 3 times on the same day and on 3 different days. The results shown in table 4 were reported in terms of relative standard deviation.

Table 4: Results of Precision (%Assay)

S.No.	Rt	Area
1	5.55	869.97
2	5.55	887.077
3	5.537	886.968
4	5.517	889.723
5	5.480	870.553
6	5.520	877.263
avg	5.5257	880.259
stdev	0.0265	8.833
%RSD	0.48	1.00

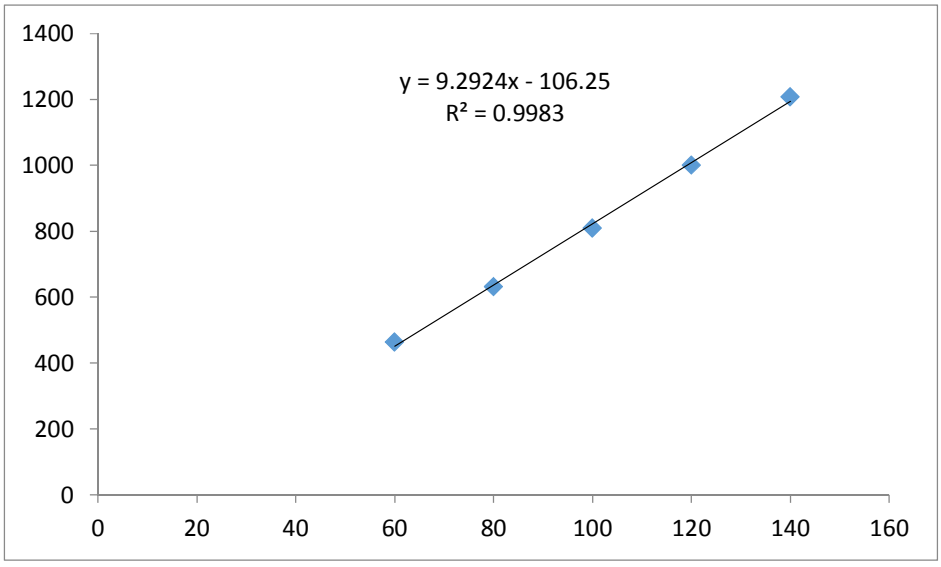

Fig 4: Linearity of Lafutidine

Linearity
Calibration graphs were constructed by plotting peak area vs concentration of Lafutidine and the regression equations were calculated. The calibration graphs were plotted over 5different linear concentrations in the range of 60-140µg/ml . Aliquots (10 ml) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n =5)]. The linearity graphs were shown in fig 4.

Limit of detection (LOD) and limit of quantitation(LOQ):
The limit of detection (LOD) and limit of quantitation (LOQ) of Lafutidine were determined by calculating the signal-to-noise(S/N) ratio of 3:1 and 10:1, respectively according to InternationalConference on Harmonization guidelines.LOD & LOQ values for Lafutidine were found to be 2.58& 6.59µg/mL respectively.

Assay of the tablet dosage form
The proposed validated method was successfully applied to determine Lafutidine in tablet dosage form. The result obtained for Lafutidine were comparable with corresponding labeled amounts. The results were tabulated in table 5.

Table 5: Results of Assay

LAFUTIDINE		
	Standard Area	**Sample Area**
Injection-1	873.254	874.316
Injection-2	875.134	871.445
Injection-3	875.177	874.919
Injection-4	811.533	875.134
Injection-5	868.506	873.263
Injection-6	875.177	875.134
Average Area	877.791	877.386
Tablet average weight	80.2 mg	
Standard weight	100.1 mg	
Sample weight	800.094 mg	
Label amount	10 mg	
std. purity	99.25	
Amount found in mg	9.96 mg	
Assay(%purity)	99.62 %	

CONCLUSION

The proposed method has advantage of simplicity and convenience for the separation and quantitation of Lafutidine which can be used for the assay of Lafutidine tablet dosage form . Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise,rapid and selective for estimation of Lafutidine in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

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REFERENCES

[1] Sundaraganapathy R,et al *Int J Pharm Pharm Sci*,**2012** Vol 5, Issue 2, 68-72
[2] Nakano M, Kitano S, *Eur J Pharmacol*.**2011** 658(2-3):236–241
[3] Shimatani T, Inoue M, Kuroiwa T, *Dig Dis Sci*.**2006** 51(1):114–120
[4] Wu L, Zhang Z, Tian Y, Li W, Xu F, Chen Y, Wei H. *J Mass Spectrom*.**2005** 40(12):1637–1643.
[5] Chena W, Lianga Y, Lia H, Xionga Y, Liua X, Wanga G, Xi L . *J Pharm Biomed Anal*.**2006** 41(1):256–260
[6] Sumithra M, Sundaram PS, Srinivasulu K . *Int J Chem Tech Res* **2011** 3(3):1403–1407
[7] Xiang-lin Z, Yun-di Q, Kai-peng L, Qingming D, Xiao L . Chin J Hospital Pharm. CNKI: SUN: ZGYZ. 0.**2005**-12-01712

[8] International Federation of Pharmaceutical Manufactures & Associations (IFPMA) (**1996**) Validation of Analytical Procedure. In: International conference on harmonization (ICH), methodology Q2(R1). Switzerland: Geneva. 6 November **1996**
[9] Ruan J, Tattersall P, Lozano R, Shah P . *Am Pharm Rev* **2006** 9(1):46–53
[10] Bakshi M, Singh S . *J Pharm Biomed Anal*. **2002** 28:1011–1040
[11] International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceutical for Human Use: Harmonized Triplicate guideline on Validation of Analytical procedures: Methodology, Recommended for Adoption at Step 4 of the ICH Process on November **1996** by The ICH Steering Committee, IFPMA, Switzerland.