International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1403-1407, July-Sept 2011

Analytical Method Development and Validation of Lafutidine in Tablet dosage form by RP-HPLC

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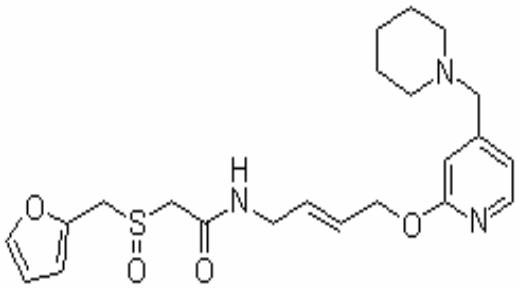
Abstract: A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of lafutidinine in tablet dosage form. Hypersil silica, C_{18} 250× 4.6 mm. 5µ. column. was used with a mobile phase containing a mixture 0.02m dihydrogen potassium ortho phosphate,0.02m dipotasium hydrogen ortho mphasphate(p^H6) and Acetonitrile and in the ratio of 30:70. The flow rate was 1.0ml/min and effluents were monitored at 215nm and eluted at 7.75min respectively. Calibration curve was plotted with a range from 27-81µg/ml. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination on lafutidinine in tablet dosage.

Key words: lafutidinine in tablet dosage, Reverse phase HPLC, 0.02m dihydrogen potassium ortho phosphate,0.02m dipotasium hydrogen ortho mphasphate.

INTRODUCTION

lafutidine 2-[(2-furylmethyl)sulfinyl]-*N*-((2*Z*)-4-{[4-(piperidin-1- ylmethyl)pyridin-2-yl]oxy}but-2-en-1-yl) acetamide are used in the treatment of anti ulcer. Structures are shown in fig 1 respectively. Antisecretory drugs are used in the treatment and prophylaxis of peptic ulcer disease some are also employed in other disorders associated with gastric hyperacidity such as gastro-oesophageal reflux disease and dyspepsia . They may be divided into Histamine H2-receptor antagonists (H_2-antagonists), which act by blocking histamine H_2-receptors on gastric parietal cells, thereby antagonising the normal stimulatory effect of endogenous histamine on gastric acid production

Fig 1: Structure of lafutidine



The literature reveals that there are some of the methods have been reported on biological method on LC–ESI–MS [1]method for the quantitation of lafutidine in human plasma Application to pharmacokinetic studies, A single LC–tandem mass spectrometry [2]method for the simultaneous determination of four H_2 antagonists in human plasma, Determination of lafutidine in human plasma [3]by high-performance liquid chromatography-electrospray ionization mass spectrometry: application to a bioequivalence study. Determination of lafutidine in human plasma by HPLC-MS[4]. As no RP- HPLC method have been reported for the determination of Sitagliptin and Metformin an attempt was made to report a simple, sensitive, validated and economic method for the determination of sitagliptin and metformin.

MATERIALS AND METHODS:

Reagents:
Acetonitrile(HPLC grade) and 0.02m dihydrogen potassium ortho phosphate,0.02m dipotasium hydrogen ortho phosphate,orthophosphate Commercial samples of tablets containing the drug were purchased from the local pharmacy.

Equipments and apparatus:
Different kinds of equipments like Analytical weighing balance, HPLC system (SHIMADZU – LC10 AT VP), Injector (Rheodyne,20µl), Sonicator, pH meter, Vacuum filter pump, Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware's were used throughout the experiment.

Chromatographic Conditions:
Analysis was carried out at 215nm using a Hypersil silica, C_{18} 250× 4.6 mm. 5µ. column dimensions at ambient temperature. The mobile phase consisted of 0.02m dihydrogen potassium ortho phosphate,0.02m dipotasium hydrogen ortho mphasphate:Acetonitrile in the ratio of (30:70, v/v) that was set at a flow rate of 1.0ml/min.

Preparation of buffer:
Dissolve 2.72gr of 0.02M Potasium dihydrogen orthophosphate,3.4gr 0.02M Dipotasium hydrogen ortho phasphate in 1000ml water to produce buffer. P^H is adjusted to 6.0 with 10% v/v Orthophosphoric acid. The pH of the solution was adjusted to 4.0±0.01 with orthophosphoric acid and then filtered through 0.45um membrane filter.

Preparation of Mobile phase:
The mobile phase was prepared by mixing 2.72gr 0.02M Potasium dihydrogen orthophosphate,3.4gr 0.02M Dipotasium hydrogen ortho phasphate in 1000ml of water (pH adjusted 6.0 with 10%v/v Orthophosphoric acid) and Acetonitrile in the ratio of (30:70). The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram

Diluent Preparation:
Use the Mobile phase as Diluent.

Preparation of standard solution:
Weigh and transfer accurately 2.72gr of 0.02m Potasium dihydrogen orthophosphate,3.4gr 0.02m Dipotasium hydrogen ortho phasphate working standard into a 100 ml volumetric flask, add about 70 ml of diluent, sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 5ml of above solution into 50 ml volumetric flask, dilute to volume with diluent and mix.

Preparation of sample solution:
For 10 mg
Transfer 1.75mg into a 250 ml volumetric flask add about 140 ml of diluents and sonicate for 15min with intermittent shaking dilute to volume with diluents and mix. Filter the solution thorough 0.45 µ nylon filter. Transfer 2ml of the above solution into a 50 ml volumetric flask, dilute to volume with diluent and mix. Filter the solution through 0.45 µ nylon membrane filter.

Method validation
The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per the earlier adopted procedure given in the experiment.

RESULTS AND DISCUSSION:

A reversed-phase column procedure was proposed as a suitable method for the determination of lafutidine in tablet dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally 0.02m dihydrogen potassium ortho phosphate,0.02m dipotasium hydrogen ortho mphasphate.buffer (pH-6),acetonitrile in the ratio of 30:70 was used. A typical chromatogram obtained by using the above mentioned mobile phase from 10µl of the assay preparation is illustrated in Fig. 1. The retention times of 7.75min, respectively. The results were discussed in Table 3.

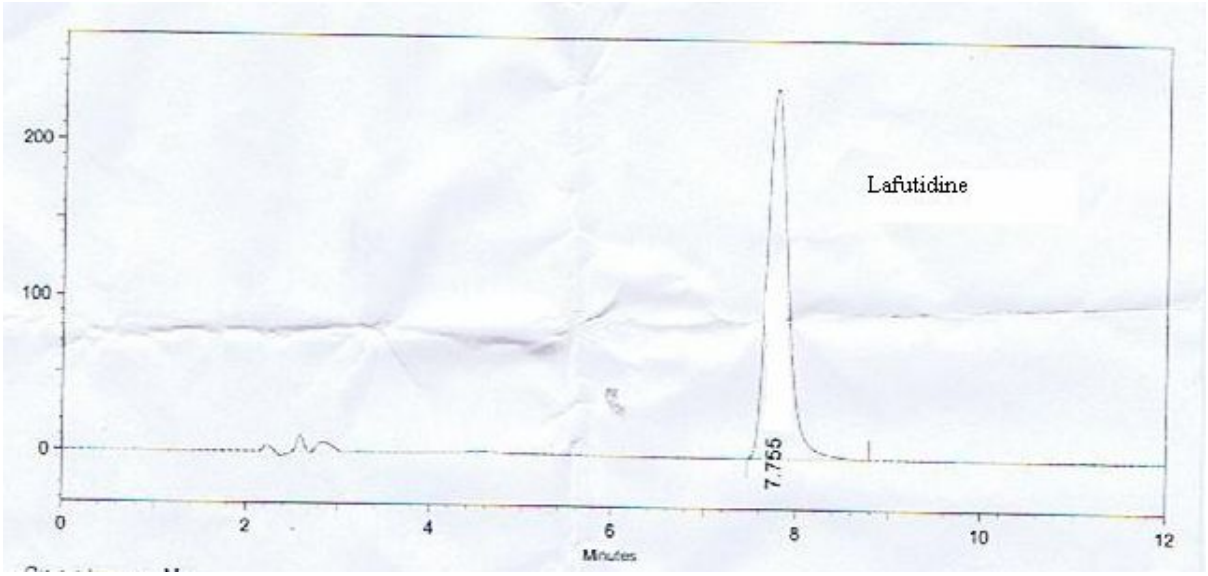


Fig. 1: A typical chromatogram showing the peaks of lafutidine in 7.75min

The linearity of the method was demonstrated over the concentration range of 27-81 µg / ml. Aliquots of 27, 40.5, 54, 67.5, and 81 µg / ml were prepared from stock solution and labeled as solution 1, 2, 3, 4 and 5 respectively. The solutions were injected in to HPLC system as per test procedure. A calibration curve was plotted for concentration v/s peak area and was given in the Fig 2.

The accuracy of the method was studied by recovery experiments. The recovery was determined at three levels, viz. 50%, 100%, and 150% of the selected concentrations. Three samples were prepared for each recovery level., The mean % recovery of the lafutidine at each level should be not less than 97.0% and not more than 103.0%. respectively (Table 1). The precision of the method was determined from lafutidine in tablet dosage form. The results are shown in (Table 2).To determine the robustness of the developed method experimental conditions were purposely altered and RSD of the peak areas of lafutidine were found not greater than 2.0 illustrate the robustness of the method.

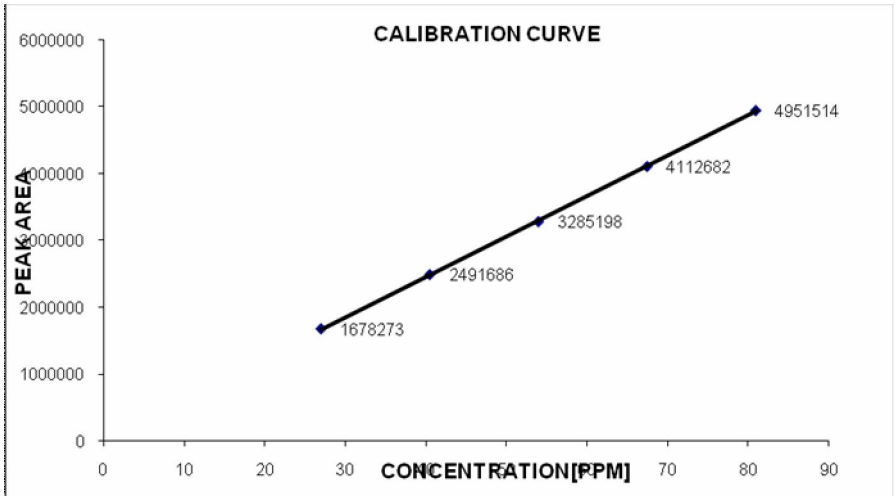


Fig 4: Linearity graph of lafutidine

Table 1: Accuracy data for lafutidine

Sample id	Concentration	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	50%	98.9	98.8	0.1	0.10121
2	50%	98.7			
3	50%	98.8			
4	100%	98.7	99.0	0.3656	0.3642
5	100%	99.4			
6	100%	98.9			
7	150%	99.2	99.2	0.15275	0.15393
8	150%	99.1			
9	150%	99.4			

*Avg of three recoveries.

Table (2): Method Precision of lafutidine by RP-HPLC

Injection number (30mcg/ml)	Retention time	Area
1	7.74	3219470
2	7.75	3251271
3	7.74	3231197
4	7.75	3223092
5	7.75	3253498
6	7.74	3220900
Avg	7.745	3233238
SD	--	0.45
%RSD	--	0.46

Table (3): Assay of lafutidine by RP-HPLC

Sample id	Concentration	Peak area	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	50%	1870250	98.9	98.8	0.10	0.10
2	50%	1875152	98.7			
3	50%	1868799	98.8			
4	100%	3751051	98.7	99.00	0.36	0.36
5	100%	3774841	99.4			
6	100%	3757991	98.9			
7	150%	5680748	99.2	99.2	0.15	0.15
8	150%	5667182	99.1			
9	150%	5695620	99.4			

CONCLUSION:

A simple RP-HPLC method was developed for the determination of lafutidine. Hypersil Silica (250 x 4.6 mm, packed with 5µm) in an isocratic mode with mobile phase 2.72gr of 0.02M Potasium dihydrogen orthophosphate,3.4gr of 0.02M Dipotasium hydrogen ortho phasphate in 1000ml water to produce buffer(p^H6), acetinitile (30:70) was used. The flow rate was 1.0ml/ min and effluent was monitored at 215 nm. The retention time was 7.75min for lafutidine.

From the linearity , it was found that the drug obeys linearity within the concentration range of 27-81µg/ml for lafutidine. From the results shown in accuracy Table 1, it was found that the percentage recovery values of pure drug were in between 98.8 to 99.2, which indicates that the method was accurate and also reveals that the commonly used excipients and

additives present in the pharmaceutical formulations were not interfering the proposed method. From the results shown in precision % RSD is less than 2%; which indicates that the proposed method has good reproducibility. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

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