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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD FOR THE ASSAY OF FAMOTIDINE IN BULK AND FORMULATIONS

T.Vijaya Bhaskara Reddy, G.Ramu , P.V.Lakshmana Rao and C.Rambabu*
Department of Chemistry, Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur-Andhra Pradesh, India
*E-mail ID: rbchintala@gmail.com

ABSTRACT

A reverse phase liquid chromatographic method (RP-HPLC) was developed to estimate the amount of famotidine in bulk and its pharmaceutical formulations. Waters- Alliance HPLC system equipped with auto sampler, ultra-violet detector and symmetry C_8 (4.6mm ID x 150mm, 3.5 µm, Make: XTerra) column were used for the quantification of the drug. Separation was carried out by using potassium dihydrogen phosphate buffer of pH=7.0 and acetonitrile in the ratio 40:60 v/v as mobile phase at a flow rate of 0.5mL/min. and the detection was carried out at a wavelength of 297 nm. The retention time, tailing factor and USP theoretical plates of famotidine were found to be 3.338min., 1.3 and 2273.9 respectively. The area of the peak was proportional to the concentration of the drug in the range 20-60 µg/mL of famotidine. The values of LOD and LOQ for famotidine were found to be 0.019 and 0.06µg/ml respectively. The mean recovery of the substance was found to be 99.8%. The developed method was found to be simple, repeatable and reproducible and hence it can be used as an alternative method in any pharmaceutical industries in the assay of famotidine.

Keywords: RP-HPLC, Famotidine, Linearity, LOD, LOD, Repeatability and Reproducibility.

INTRODUCTION

Famotidine (FMD) is a histamine H_2-receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of peptic ulcer disease (PUD) and gastro esophageal reflux disease (GERD/GORD). It is commonly marketed by Johnson & Johnson/Merck under the trade names Pepcidine and Pepcid and by Astellas under the trade name Gaster. Unlike cimetidine, the first H_2 antagonist, famotidine has no effect on the cytochrome P450 enzyme system, and does not appear to interact with other drugs[1]. Famotidine was developed by Yamanouchi Pharmaceutical Co.[2]. It was licensed in the mid-80s by Merck & Co.[3], and is marketed by a joint venture between Merck and Johnson & Johnson. Famotidine is given to surgery patients before operations to prevent postoperative nausea and to reduce the risk of aspiration pneumonitis. Famotidine is also given to some patients who take NSAIDs, to prevent peptic ulcers[4]. It serves as an alternative to proton-pump inhibitors[5] .Famotidine has also been used in combination with an H1 antagonist to treat and prevent urticaria caused by an acute allergic reaction. The IUPAC name of the drug is 3-([2-(diaminomethyleneamino)thiazol- 4-yl]methylthio)- *N′*-sulfamoylpropanimidamide, with molecular formula and molecular weight $C_8H_{15}N_7O_2S_3$ and 337.449 g/mol respectively. The molecular structure of the drug is given in Fig.1

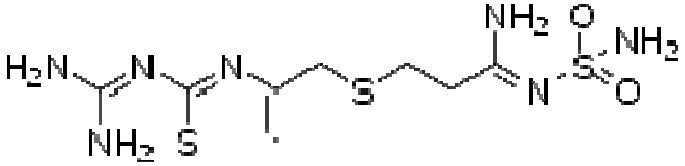

Fig.-1: Molecular structure of Famotidine

Literature survey reveals High Performance Liquid Chromatographic (HPLC)[6-8] and High Performance Thin Layer Chromatographic (HPTLC)[9-11] methods for determination of FMD in tablet dosage form. Spectrophotometric methods [12-15] for quantification of FMD are also reported. Spectrophotometric methods have been reported for determination of FMD in combination with other drug in tablet dosage form[16]. A spectrophotometric and spectrofluorimetric method [17] were developed for the determination of FMD.

Table-1: Precision of the proposed method

Injection	Area	Area
	Intraday precision	Inter day precision
Injection-1	4137672	4137672
Injection-2	4152307	4152307
Injection-3	4162984	4162984
Injection-4	4199069	4199069
Injection-5	4193409	4193409
Average	4169088	4169088
Standard Deviation	26439.6	26439.6
%RSD	0.63	0.63

Table-2: Linearity of the peak area against amount of the drug

S.No	Concentration (µg/ml)	Area
1	20	2102553
2	30	3098117
3	40	4124711
4	50	5025966
5	60	6024874
Slope Intercept Correlation Coefficient		10010 59374 0.9990

Table-3: Accuracy of the proposed method

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2095762	5.0	5.09	101.8%	99.8%
100%	4078121	10.0	9.91	99.1%	
150%	5995394	14.8	14.5	98.4%	

Table-4: Study of effect of small variation flow rate and composition of mobile phase (robustness)

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.4	2308.8	1.3
2	0.5	2271.4	1.3
3	0.6	2233.9	1.2

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2083.6	1.2
2	Actual	2271.4	1.3
3	10% more	2061.5	1.3

Table-5: Study of degradation of the drug

Degradation Parameter	Peak Area	Peak Area of Standard	% of Recovery	% of Degradation
0.1M HCl	3840607.000	4129685.000	92.99999879	7.00
0.1 M NaOH	3758013.000	4129685.000	90.99999152	9.00
Thermal	3592826.000	4129685.000	87.00000121	13.00
Peroxide	3510232.000	4129685.000	84.99999395	15.00

EXPERIMANTAL

Instrumentation

Waters-Alliance HPLC system equipped with auto sampler, binary gradient pump, and dual wavelength UV-Visible detector was used to for the determination. An analytical column; Symmetry C_8 (4.6 mm ID x 150mm, 3.5 µm, Make: XTerra) was used in the analysis. Chromatographic software Empower -2 was used for data collection and processing. Elico-SL159 model, 2nm high resolution, double beam, 1cm length quartz coated optics and wavelength range190-1100nm is used for measuring absorption spectrum

Materials and Methods

Famotidine pure drug was gifted by Dr.Reddy's Laboratories Ltd., Hyderabad. The commercially available formulations of famotidine were purchased from the local market. The water of HPLC was prepared by double glass distillation and filtration through 0.45 mm filters. Acetonitrile of HPLC grade was obtained from E.Merck. (India) Ltd., Mumbai. Potassium dihydrogen phosphate and sodium hydroxide of analytical grade are purchased from Qualigens Fine Chemicals Ltd., Mumbai. About 7.0 grams of potassium dihydrogen phosphate was weighed accurately, transferred into a 1000mL beaker and dissolved in 500mL of HPLC grade water. The solution was sonicated for 30min., degassed and then made to total volume. The pH of the resulting solution was adjusted to 7.0 by adding dilute sodium hydroxide solution and filtered through 0.45µm membrane filter. The mobile phase was prepared by adding of 600mL acetonitrile to 400mL of 0.7%potassium dihydrogen phosphate buffer of pH 7.0; the solutions were mixed well, degassed for 30min. and filtered through 0.45µm membrane filter.

Preparation of standard and sample solutions

Stock solution of the famotidine was prepared by dissolving accurately weighed 10mg of Famotidine standard in 7mL of mobile phase in a 10 mL volumetric flask, sonicated and made up to the mark. Working standard solution of 40µg/mL was prepared by transferring about 4.0 ml of the above stock solution into a 100ml volumetric flask and dilute up to the mark with mobile phase, sonicated and filtered through 0.45µm filter. A series dilute solutions were prepared in similar manner and transferred into an auto sampler vial for analysis. The column was allowed to equilibrate for 30min. by allowing mobile phase at a flow rate of 0.7 mL/min. prior to the analysis. Famotidine working standard (40µg/ml) solution was used to evaluate system suitability parameters. 20µl of standard solution, blank, five replicate injections of standard solution and sample solution were separately injected and monitored at 215 nm. The absorption spectrum shown in Fig.2 indicate that the famotidine absorbs UV radiation in considerable extent in the range of 210-220nm hence wavelength 215nm is chosen for the analysis.

Five tablets of famotidine were accurately weighed and finely powdered in a mortar. An amount of tablet mass equivalent to 10mg was transferred to a 10 mL volumetric flask and dissolved in 7 mL of mobile phase. Then the flask was placed in ultrasonic bath for 15 min. The resulting suspension was diluted to volume with mobile phase and then filtered through 0.45µm membrane. Further three different concentration solutions (i.e. 50%, 100% and 150%) of the target concentration were prepared and the percent of recovery was studied. The analysis was carried out under the isocratic conditions with a flow rate of 0.8ml/min. and injection volume 20µl. The data were acquired at 239 nm and processed by use of Empower software for data handling system. A mixture of buffer and acetonitrile in the ratio 35:65 (v/v) was used as diluents in the preparation of analytical solutions. Famotidine working standard (40µg/ml) solution was used as system suitability solution. 20µl of system suitability solution, blank, five replicate injections of diluted standard solution and sample solution were separately chromatogramed.

Method Validation

Precision of the method was studied for repeatability and intermediate precision. Repeatability was determined by analyzing five separate famotidine sample solutions and the %R.S.D.was found to be 0.630%. The intermediate precision of the method was determined on five separate sample solutions prepared from same lot by spiking by different days. The %R.S.D was evaluated and found to be 0.460% which was within the acceptance criterion of NMT 10% R.S.D. The results are presented in Table-1. Accuracy of the method was determined by analyzing Famotidine sample spiked at three different concentration levels 50, 100 and 150% of each in triplicate at the specified limit. The percent of recovery at each level was calculated and found to be within the acceptable limits. The mean recovery of the drug was found to be 99.8% and the data was given in Table 3

The plot of peak areas versus concentration of famotidine was plotted and found to be linear. The linear plot was given in Fig.5. The linear regression analysis was carried out to calculate slope, intercept and correlation coefficient and the results were presented in Table 2. The limit of detection (LOD) and limit of quantitation (LOQ) were determined for famotidine from the standard deviation of the peak area and slope of the linearity data. The values of LOD and LOQ for famotidine were found to be 0.019 and 0.060µg/ml respectively. The results were depicted in Table 2. To evaluate the robustness of the method, the influence of small and premeditated alteration of analytical parameters such as flow rate and percent of composition of the mobile phase on the quantification of the drug substance and selectivity was studied. The studies indicated that no considerable effect on the determination of the drug. Therefore the test method is robust for the quantification of the drug. The results were presented in Table 4. The percent of drug degraded in the presence of acid, base, thermal and peroxide conditions were studied. The drug standard was exposed to 0.1NHCl solution, 0.1N NaOH and 1% peroxide solutions for 48 hours at room temperature and exposed to 45°C for about 36 hours. The amount of drug recovered or degraded is calculated by comparing the area of the standard with that of the area of the degraded sample. The results are presented in Table 5.

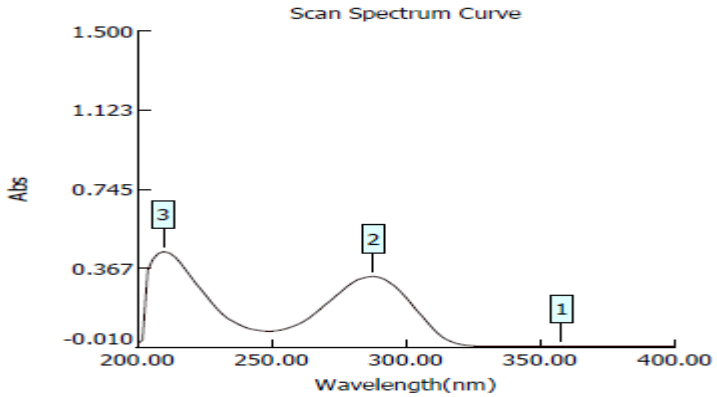

Fig.-2: Absorption spectrum of famotidine

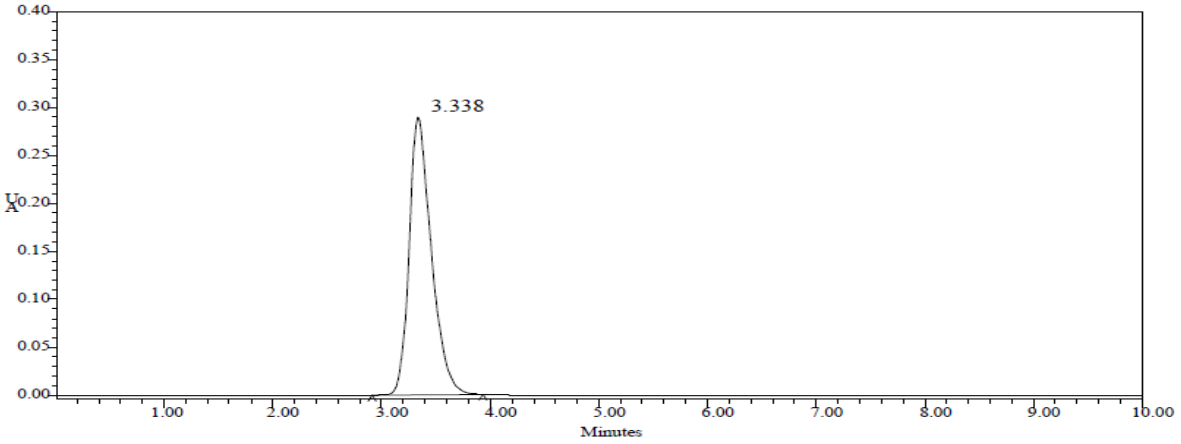

Fig.-3: A typical chromatogram of famotidine standard

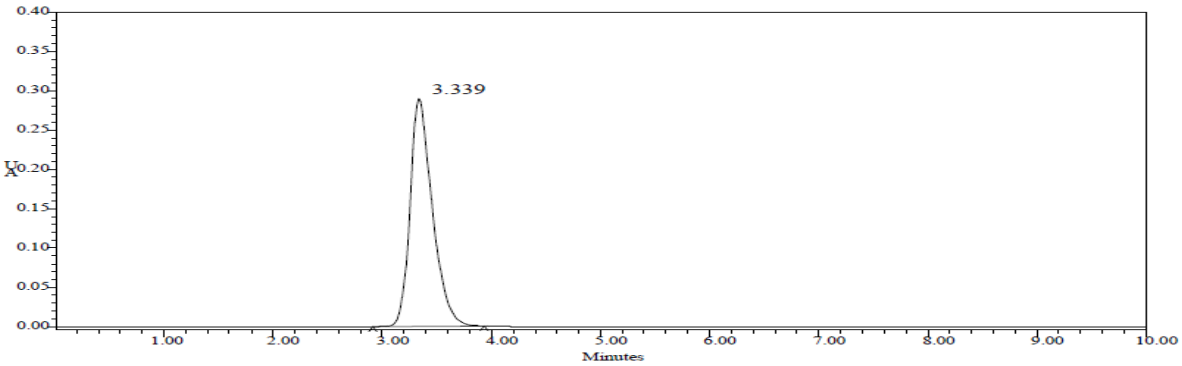

Fig.-4: A typical chromatogram of famotidine formulation

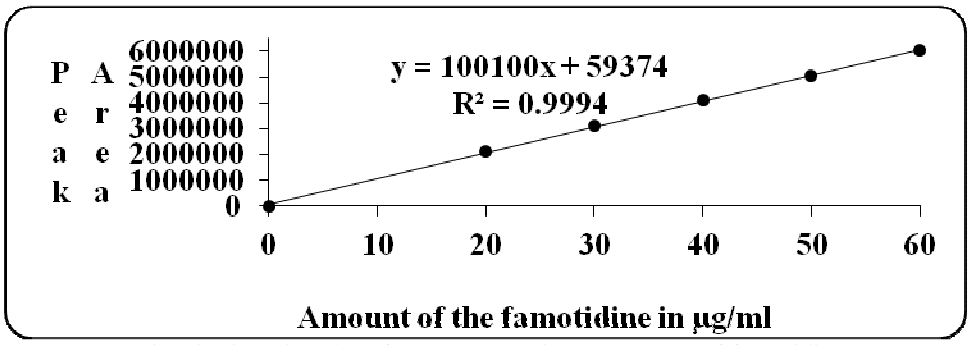

Fig.-5: Linearity plot of peak area against to amount of famotidin

RESULTS AND DISCUSSION

The system suitability parameters such as tailing factor (1.3) and number of theoretical plates (2273) are found to be within the limits and the retention time of the component was found to be 2.256min. A typical chromatogram for the standard and sample were presented in Fig.3 and Fig.4 respectively. The intra-day precision or inter-day precision of a method was expressed in terms of statistical parameters such as standard deviation and %RSD, calculated for five replicate measurements and found to be less than 2.0. Inter-day precision of the method was determined by carrying out the experiment on different days using same instrument and same column under similar chromatographic conditions. The results are presented in Table-1. The proposed method was linear in the range of concentration 20-60µg/ml (Fig.5). The correlation coefficient, slope and intercept were presented in Table-2.The accuracy of the method was determined from recovery experiments. The recovery studies were carried out at three different

concentration levels (50%, 100% and 150% of target concentration). The percentage recovery of the drug at three different concentration levels and the mean percent of recovery are found to be within the specified limits and presented in Table-3. Robustness of the proposed method is checked by making slight deliberate change in the flow rate and mobile phase composition is made to evaluate the impact on the method. The results are summarised in Table-4. A study on degradation of the drug was conducted and found to be negligible.

CONCLUSIONS

The developed isocratic RP-HPLC is found to be precise and accurate as indicated by repeatability and recovery studies. Recovery studies are performed at 50%, 100% and 150% concentration levels are found to be within the limits mentioned as per ICH Guidelines. The proposed method was found to be simple, sensitive and robust. Therefore the method can be used for routine analysis in quality control.

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