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ORIGINAL ARTICLE

Analysis of Famotidine in API and Formulation using UV and HPLC

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ABSTRACT

Famotidine is histamine-2 blocker. These H2 antagonists are competitor of histamine. Hydrochloric acid is secreted by the Parietal cells that are present in epithelial cells of stomach and stifle the secretion of acid. We have developed the Spectrophotometric technique for the analysis of Famotidine. A drug sample dissolved in water to make Famotidine solution. In the same way, different dilutions were made. Absorbance was measured at 265nm and the assay was performed at 5 different dilutions of 100ppm. Percent assay and regression equation was also obtained to calculate further availability of drug.

A simple, efficient and least time-consuming method was validated by HPLC. Company Shimadzu LC 20AT model was used containing 150 mm column. Mobile phase having ratio of (80:20, v/v) methanol: water was used and pH was adjusted to 3.30. Analyte concentrations were determined at wavelength 265nm using a UV-detector. All analyses were carrying out at room temperature (25 ± 2C). Famotidine was separated in formulations in approx. 5.0 min, linear calibration curves were obtained at concentration range from 100–25ppm. 0.9998 of correlation coefficients with an average recovery above 99.91% was also detected. Also, retention time (Rt) recognition of analytes from different dosage forms shows the stability and specificity of the developed method.

INTRODUCTION

Famotidine is H-2 blocker. These H_2 antagonists are competitor of histamine [1] (Figure **1**). In normal individuals and hyper secretors, Famotidine restrained basal gastric secretion. Antisecretory effect occurred within one hour after oral administration, it is dose-dependent drug, and its effect remains one to three hours. At doses of 20 to 40mg its inhibit secretion 10 to 12 hours [2,3].

Famotidine is incompletely absorbed in orally form and having bioavailability only forty to forty-five percent. It undergoes first-pass metabolism. Peak plasma levels of oral dose come about in 1 to 3 hours elimination half-life of Famotidine is above 3 hours. Famotidine is excreted by renal excretion to sixty-five to seventy percent. Twenty-five to thirty percent of an oral dose and sixty-five to seventy percent of an IV dose are unchanged in urine [3].

Numerous methods were reported for analysis of Famotidine by UV [4-5] and HPLC [1, 2] but there is no method using same concentration and diluents for both HPLC and UV method at a time. This method is very simple, cheap and less time consuming.

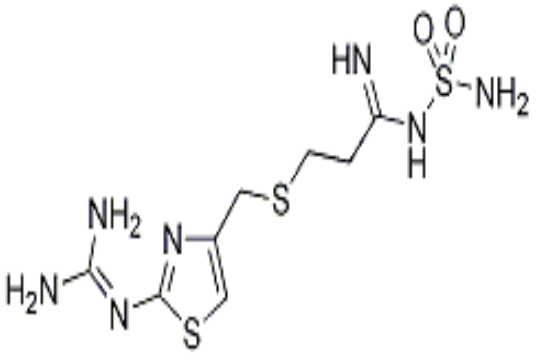

Figure 1. Structure of Famotidine.

METHODOLOGY

Famotidine was obtained by Pakistan and used as reference standard. Methanol was purchased by VWR International, Pakistan. Membrane filter (polyethylene, 0.45µm pore size) was used for purification of all solvents and also degassed before use by ultrasonication (Ultrasonicator, Pakistan). All solvents were used of analytical grade.

Instrumentation and chromatographic conditions

Shimadzu (model 1800) UV visible double beam spectrophotometer was used for the analysis of spectra. For the assay water and methanol as a ratio of 90:10ml is used.

High performance liquid chromatograph Shimadzu LC-20 AT were used, outfitted with UV-detector SPD-10A, connected with Shimadzu LC-20 for integration of chromatograms. Column for HPLC are of MEDITERRANEA SEA 18 of dimension 150mm 4.6mm was used. All performance was carried out at room temperature (25± 2C). At ratio of (80:20v/v) methanol: water is used as a mobile phase and adjusted pH at 3.3. Set flow rate at 1.0ml/min and 10µL was volume of injection was used. All solutions were sonicated for 30-minute prior using and filtered membrane filter of 0.45-micron, UV spectrum was carried out at 265nm for Famotidine.

Calibration curves

Standard amount of 10mg is accurately weight of Famotidine and then transferred to a 100 ml flask. Required volume was makeup with the mobile phase. All analytes were sonicated before use and then filtered through a membrane filter pore size 0.45 µm. Now concentration was achieved is 100ppm. Five different concentrations of Famotidine (6.25, 3.125, 1.56, 0.78 & 0.39ppm) were attained of standard solution. 20µL of each solution was introduced in the chromatographic system and peak areas were plotted against concentrations.

Method validation

We have to validate method to authenticate that the procedure that is analyzed for a specific test is appropriate for its intended use. Linearity is defined as mathematical transformation that is proportional to the analyte concentration in the given range. It is used to calculate the coefficient correlation and calibrating the curves. Furthermore, method precision was used to assess by interday and intraday repeatability. The interday and intraday deviation for Famotidine was carried out at different concentration (6.25, 3.125, 1.56, 0.78 & 0.39ppm) and expressed in terms of %RSD.

Furthermore, robustness was also carried out by minor intentional alterations in mobile phase ratio. Different ratios of mobile phase were used but finest peak achieved in methanol: water (80:20, v/v ± 1mL) ratio at pH at (3.2 ± 0.2) and possibly slight variation in the flow rate i.e., 1.0± 0.2mL/min were attempt in the recommended method.

Standard stock solution

10mg famotidine accurately weigh and transferred to 100ml volumetric flask and makeup volume with methanol.

Sample preparation

10 tablets of Famotidine were weighed and crushed. By calculating their average weighed 10 mg equivalent weight of Famotidine is then transferred into a graduating flask and makeup volume up to 100ml. The solutions were also sonicated for about 30min.

Dilutions preparation

Five dilutions of 6.25, 3.125, 1.56, 0.78 & 0.39ppm were made from 100ppm sample Famotidine solution for both UV and HPLC method.

Procedure for UV analysis

100ppm of standard and sample were prepared and take the absorbance at about 265nm by 1cm cell at the maximum wavelength using a spectrophotometer.

Procedure for HPLC analysis

First 100ppm standard and sample solutions were prepared in 100ml volume of methanol and water at a proportion of 80:20, v/v ± 2ml. Linearity is assessed to find out the coefficient correlation and calibrating curves. Then, method was précised by interday and intraday repeatability. Precision was carried out at different concentration levels that are 75, 125 250ppm and expressed in terms of %RSD. Moreover, robustness was also performed by slight intentional adjustment in the mobile phase ratio. Using mobile phase at the ratio of methanol: water (80:20, v/v ± 2ml) and maintain its pH to 3.3± 0.2 and also perform insignificant alterations in the flow rate 1.0± 0.2ml/min were attempted in the proposed method.

RESULTS & DISCUSSION

Famotidine formulation was analyzed by using spectrophotometer. Table **1** shows different concentration and absorbance of famotidine formulation dilutions.

Table 1. Absorbance of sample and standard of Famotidine v/s Concentration.

Conc.	Absorbance of Sample	Absorbance of Standard
6.25	0.627	0.536
3.125	0.351	0.251
1.56	0.173	0.121
0.78	0.076	0.05
0.39	0.052	0.022

Method development of the optimum mobile phase

HPLC technique was used to analysis and method development for the quantitative purpose of Famotidine. Active drug and formulation analytes were introduced into HPLC and run different dilution solution. Primarily methanol–water and methanol-water-acetic acid was tried in different proportions. Then mobile phase having ratio 70:30 v/v (methanol: water) show good results with retention time 2.52 min but peak was not sharp because of tailing. Changing concentration ratio at 80:20, v/v methanol: water gets better peak. As a final point, the mobile phase contains methanol: water (80:20, v/v) ratio gave a distinct and well-defined peak at retention time 5 min (as shown in Figure **2**).

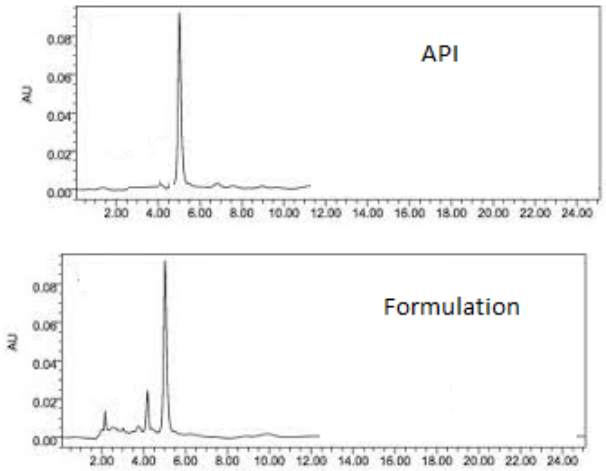

Figure 2. Chromatogram of Famotine in API and Formulation.

Method validation

For the investigation of drug, a number of preliminary tests were conducted to ensure the validity of products. Parameter that we should focus is optimum pH, fixed proportion of solvents, optimum concentration of vehicles and wavelength detection. Flow rate was also selected at 1ml/min after preliminary tests.

Calibration curves

Famotidine showed a good linear relationship at 265nm at different concentration range relating to the peak area (Table **1**). The observed equation was found as; y=30597x=2E=06 of the straight line having R2-value higher than 0.996. Linearity values explain that the concentration is proportional to the of the analyzed substance concentration. System suitability parameters are given in Table **2**.

Table 2. System suitability parameter.

Parameter	Active
Ret. Time	2.296
Height	1960334
Resolution	1.102
Theoretical Plates (N)	5452.256
Capacity Factor (K')	1.95

Accuracy

Accuracy is defined as the nearness of test results attained by that process or method to the true value. Percent recoveries of response factor (area/concentration) were calculated. Famotidine accuracy values (<99.376) of active and for formulation its values are (<99.36) for integrand intra-day variations implying an admirable accuracy of the developed HPLC method. Accuracy results are shown in Table **3**, and it is also shown that our prescribe method is accurate and within the expected recovery range.

Recovery studies

Assay method accuracy was assessed in five different concentrations i.e. 6.25, 3.125, 1.56, 0.78 & 0.39ppm. From the slope and y-intercept %RSD and percentage recoveries were analyzed and calibration curve was obtained. The % recovery values acquired were among the ranges of 99.376 -100.01333% confirming accuracy of the developed method.

Robustness of the method

There was no chief difference in retention time and area when we deliberately change in mobile phase constituents as little as ±2ml, so no changes were observed in chromatogram as well as in retention time or area. But when we change in flow rate from 1ml/min to 0.8 ml/min there was a slight delay in retention time. While in slight changes in pH that is ±0.2 units of the mobile phase there is slight increase in the area of chromatogram but no difference was observed in retention time. Low values of SD and % recovery values with insignificant retention time were obtained after introducing slight deliberately changes show that the Famotidine robustness method was accurately developed (follow Table **5**).

Table 3. Accuracy of active and formulation by HPLC.

Drugs	Conc injected	Conc found	Inter-day	Intra-day
	µg mL_1	µg mL_1	percent (%) Recovery	percent (%) Recovery
Standard	75	75.01	100.013333	100.013
	125	124.22	99.376	99.54
	250	250.012	100.0048	100.44
Formulation	75	72.01	100.013345	100.015
	125	133.23	99.452	99.36
	250	255	100.0052	100.52

Precision

At three different concentration levels of 75, 125 and 250ppm show peak area and %RSD of famotidine active in the range of 0.000024 to 0.0000043 and for formulation it values are 0.0000150 to 0.0000026 for inter and intraday respectively as shown in Table **4**.

Table 4. Precision of active and formulation.

Conc	Active %RSD		Formulation %RSD	
	Inter-day	Intra-day	interday	intraday
75	0.000024	0.00004	0.000015	0.00002
125	0.000009	0.000019	0.000001	0.00005
250	0.00001	0.00004	0.000005	0.000008

Purpose of the developed method analysis of Famotidine in tablets

A single peak of Famotidine samples was shown in the chromatogram that was obtained from tablets. No intrusion from the excipients find in tablets. %RSD value was low specified the appropriateness of this method for practical analysis of Famotidine in conventional method.

Table 5. Robustness of the method.

	Level	Resolution	Ret. Time	Capacity Factor (K')
1: Flow rate				
0.8 sec	-0.2	1.101	2.288	1.95
1 min	0	1.012	2.296	1.85
1.2 min	0.2	1.011	2.294	1.65
SD		0.051675268	0.00416333	0.152752523
2: pH of mobile phase				
3	-0.2	1.001	2.27	1.485
3.2	0	1.1	2.845	1.495
3.4	0.2	1.012	2.88	1.515
SD		0.054261711	0.34252737	0.015275252
3: Wavelength in nm				
260nm	-5	1.01	2.288	1.77
265nm	0	1.021	2.296	1.95
270nm	5	1.01	2.294	1.615

CONCLUSION

Analysis of Famotidine in API and Formulation using UV and HPLC was carried out; and observed a good linear relationship. The correlation coefficient of Famotidine drug was found to be > 0.999 while coefficient relation value is well and within the limit.

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