

SIMPLE AND SENSITIVE UV SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF FAMOTIDINE IN TABLET FORMULATIONS

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Abstract

Three new, simple, cost effective, sensitive and accurate UV spectrophotometric methods are described for the determination of famotidine (FMT) in bulk drug and in tablets. The methods are based on measurement of absorbance of FMT in 0.1 M NaOH solution at 286 nm (method A), 0.1 M HCl solution at 265 nm (method B) and in 0.1 M H₂SO₄ solution at 265 nm (method C). Beer's law is obeyed over the concentration ranges 0.63 - 15.0 µg/mL for method A and 1.25 - 30.0 µg/mL for methods B and C, respectively. The molar absorptivity values are calculated to be 2.14×10^4 L/mol/cm (method A), 1.18×10^4 L/mol/cm (method B) and 1.22×10^4 L/mol/cm (method C) and the corresponding Sandell sensitivity values are 0.016, 0.029 and 0.028 µg/cm². The limits of detection (LOD) and quantification (LOQ) are also reported. Intra-day and inter-day precision, and accuracy of the method were established according to ICH guidelines. The methods were successfully applied to the determination of FMT in tablets and the results were statistically compared with those of the reference method by applying the Student's t-test and F-test. No interference was observed from the common excipients added to tablets. The accuracy and validity of the method was further ascertained by performing recovery experiments *via* standard-addition procedure.

Rezumat

Scopul studiului a fost dezvoltarea și validarea a trei metode UV pentru determinarea famotidinei ca substanță și din comprimate.

Avantajele noilor metode sunt simplitatea și rapiditatea procedurilor și costul scăzut al reactivilor și a aparaturii folosite.

Metodele nou descrise se pot utiliza în controlul calității și în analizele de rutină în industrie sau laboratoare farmaceutice, având în vedere precizia și acuratețea lor, precum și lipsa interferențelor excipienților uzuali în determinarea famotidinei din comprimate.

Keywords: UV spectrophotometry, famotidine, tablets.

Introduction

Famotidine (FMT), 3-[2-(diaminomethyleneamino) thiazol-4-ylmethylthio]-N-sulfamoylpropionamide (Fig. 1), is a histamine H₂-receptor antagonist (H₂-RA) which competitively inhibits the action of

histamine on the H₂-receptors of parietal cells and thereby reduces the gastric acid secretion under daytime and nocturnal basal conditions as well as secretion stimulated by food and pentagastrin. It is used for the short term treatment of duodenal ulcer and treatment of pathologic hypersecretory conditions like Zollinger-Ellison syndrome [1]. FMT is official in the United States Pharmacopoeia [2] and in the British Pharmacopoeia [3], and both describe the non-aqueous titration with potentiometric end point detection.

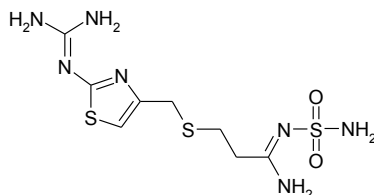


Figure 1

Structure of famotidine

Several techniques including HPLC [4], HPTLC [5], capillary electrophoresis [6], differential pulse voltammetry [7], potentiometry [8], polarography [9], chemiluminescence spectrometry [10] and spectrofluorometry [11] have been employed for determining FMT in pharmaceutical dosage forms. Some of these methods have enough sensitivity to determine lower concentrations of drug; however, many of these techniques are deficient in simplicity, cost-effectiveness and accessibility.

Spectrophotometry is characterized by its speed and simplicity, accuracy and inexpensive instrument needed, and hence it is an important alternative to other analytical techniques with clear advantages in terms of cost of analysis. The most widely used technique for the assay of FMT in pharmaceutical formulations has been visible spectrophotometry and methods based on charge-transfer complexation [12, 13], ion-pair complexation [14], condensation reaction [15], redox [16-18], bromination [19] reactions have been reported by several researchers. However, most of the above visible spectrophotometric methods suffer from at least one disadvantage such as poor sensitivity, poor selectivity, use of expensive reagents or heating step, narrow linear range, strict pH control and extraction step.

There is only one report on the use of UV-spectrophotometry for the determination of FMT in pharmaceuticals. R Sahu *et. al.* [20] used dimethylformamide (DMF)/0.1 N HCl (1:3) as solvent and measured the absorbance at 267 nm.

In the present study, three new, simple, sensitive and efficient UV-spectrophotometric methods were elaborated for the determination of FMT in pure form and in tablet dosage form. The present methods are advantageous compared to the previously reported UV-spectrophotometry method in terms of sensitivity.

Materials and Methods

Apparatus: All absorbance measurements were performed on a Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer provided with 1-cm matched quartz cells.

Materials and reagents: All chemicals and reagents used were of analytical or pharmaceutical grade and distilled water was used throughout the experiment.

Standard FMT solution: Pharmaceutical grade FMT sample was kindly provided by Cipla India Ltd, Mumbai, India, and was used as received. Standard FMT solution (500 µg/mL) was prepared by dissolving the calculated quantity of pure drug in 0.1 M NaOH (method A), 0.1 M HCl (method B) and in 0.1 M H₂SO₄ (method C) and diluted to 25 µg/mL (method A) and 50 µg/mL (methods B and C) using the respective solvents.

Two brands of tablets containing FMT, Topcid-20[®] (Torrent Pharmaceuticals Ltd., H. P, India) and Famocid-20[®] (Sun Pharmaceuticals Industries, Jammu, India), used in the investigation were purchased from local commercial sources.

Sodium hydroxide (0.1 M): Prepared by dissolving the calculated quantity of the chemical (Merck, Mumbai, India) in 250 mL of water.

Hydrochloric acid (0.1 M): Concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.18) was appropriately diluted with water to get the required concentration.

Sulphuric acid (0.1 M): Concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.84) was appropriately diluted with water to get the required concentration.

Procedures

Method A: Varying aliquots (0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL) of 25 µg/mL standard FMT solution in 0.1 M NaOH were taken into a series of 10 mL volumetric flasks, the content was diluted to the mark with 0.1 M NaOH and mixed well. The absorbance of each solution was measured at 286 nm versus 0.1 M NaOH.

Methods B and C: Varying aliquots (0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL) of 50 µg/mL standard FMT solution in 0.1 M HCl (method B) and 0.1 M H₂SO₄ (method C) were taken into a series of 10 mL volumetric flasks,

the content was diluted to the mark with the corresponding solvents and mixed well. The absorbance of each solution was measured at 265 nm in both methods A and B *versus* the respective solvents.

Calibration graphs were made by plotting the increasing absorbance values *versus* concentrations of FMT. The concentration of the unknown sample was read from the respective calibration graph or deduced from the regression equation derived using the Beer's law data.

Procedure for tablets

Twenty tablets were weighed accurately and ground into a fine powder. An accurately weighed amount of the powdered tablet equivalent to 50 mg of FMT was transferred to a 100 mL standard flask and shaken with 60 mL of 0.1 M NaOH (method A), 0.1 M HCl (method B) or 0.1 M H₂SO₄ (method C) for about 20 min, then made up to the mark with the respective solvents, mixed and filtered using a Whatman No. 42 filter paper. The filtrate containing 500 µg/mL FMT was appropriately diluted with the respective solvents to obtain the working concentrations of 25 µg/mL for method A and 50 µg/mL for method B and method C and the assay was completed by following the recommended procedures.

Procedure for placebo blank

A placebo blank of the composition: talc (10 mg), starch (5 mg), acacia (5 mg), methyl cellulose (10 mg), sodium citrate (5 mg), magnesium stearate (10 mg) and sodium alginate (5 mg) was made and the solution was prepared as described under 'Procedure for tablets', and then subjected to analysis using the procedures described above.

Procedure for synthetic mixture

To the placebo blank of the composition described above, 50 mg of FMT was added and homogenized, transferred to a 100 mL standard flask and the solution was prepared as described under 'Procedure for tablets'. The synthetic mixture solution (500 µg/mL in FMT) was then diluted with the respective solvents to obtain working concentrations of 25 µg/mL for method A and 50 µg/mL for method B and method C and then subjected to analysis. The analysis was used to study the interferences of excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

Results and Discussion

FMT solution in 0.1 M NaOH (method A), 0.1 M HCl (method B) and 0.1 M H₂SO₄ (method C) exhibited absorption peaks at 286 and 265 nm for method A and methods B and C, respectively (Figure 2) and the

absorbance at these wavelengths was found to be linearly dependent upon the concentration of drug. In all the cases, the corresponding blank solutions showed negligible absorbance. Therefore these wavelengths were used as analytical wavelength throughout the investigation.

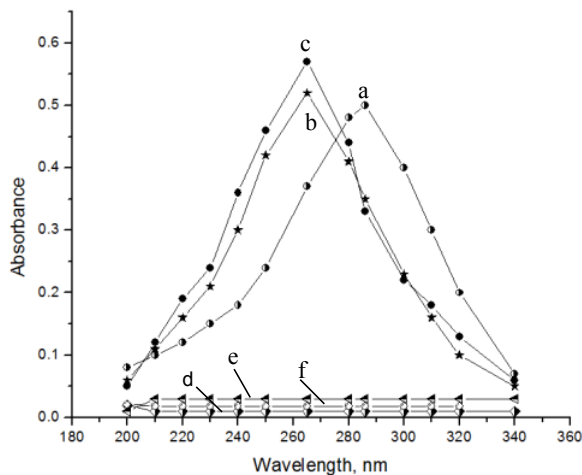


Figure 2.

Absorption spectra (a= method A, 7.5 µg/mL FMT, b= method B, 15 µg/mL FMT, c= method C, 15 µg/mL FMT, d=blank for method A, e=blank for method B and f=blank for method C)

Method Validation Procedures

The proposed methods were validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and Sensitivity

Under optimum conditions a linear relation was obtained between absorbance and concentration of FMT in the range of 0.63 - 15.0, 1.25 - 30.0 and 1.25 -30.0 µg/mL for method A, method B and method C, respectively. The calibration graph is described by the equation:

$$Y = a + b X$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in µg/mL) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table I. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification are calculated as per the current ICH guidelines [21] They are compiled in Table I and show the excellent sensitivity of the proposed method. The limits of detection

(LOD) and quantification (LOQ) were calculated according to the same guidelines using the formula:

$$\text{LOD}=3.3\sigma/s \text{ and } \text{LOQ}=10\sigma/s$$

where “ σ ” is the standard deviation of five reagent blank determinations and “ s ” is the slope of the calibration curve.

Table I.
Sensitivity and Regression Parameters

Parameter	Method A	Method B	Method C
λ_{max} , nm	286	265	265
Linear range, $\mu\text{g/mL}$	0.63-15	2.5-30	2.5-30
Molar absorptivity(ϵ), L/mol/cm	2.14×10^4	1.18×10^4	1.22×10^4
Sandell sensitivity ^a , $\mu\text{g/cm}^2$	0.016	0.028	0.029
Limit of detection (LOD), $\mu\text{g/mL}$	0.11	0.18	0.13
Limit of quantification (LOQ), $\mu\text{g/mL}$	0.29	0.54	0.40
Regression equation, Y^b			
Intercept (a)	0.011	0.008	0.0003
Slope (b)	0.06	0.036	0.035
Standard deviation of a (S_a)	0.005	0.043	0.011
Standard deviation of b (S_b)	0.0004	0.002	0.0004
Regression coefficient (r)	0.999	0.999	0.999

^a Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and $l = 1 \text{ cm}$.

^b $Y=a+bX$, Where Y is the absorbance, X is concentration in $\mu\text{g/mL}$, a is intercept, b is slope.

Precision and accuracy

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of the FMT were prepared and

analyzed in seven replicates. The analytical results obtained from this investigation are summarized in Table II. The low values of the relative standard deviation (% R.S.D) and percentage relative error (% R.E) indicate the high precision and the good accuracy of the proposed methods. The percentage relative error was calculated using the following equation:

$$\%R.E = \left[\frac{\text{found} - \text{taken}}{\text{taken}} \right] \times 100$$

Table II.
Evaluation of intra-day and inter-day accuracy and precision

Method	FMT taken µg/mL	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=7)		
		FMT found µg/mL	%RE	%RSD	FMT found µg/mL	%RE	%RSD
Method A	3.0	3.06	2.00	0.95	2.93	2.33	1.02
	6.0	5.87	2.17	1.03	5.84	2.67	1.14
	9.0	8.85	1.67	1.09	8.79	2.33	1.25
Method B	5.0	5.05	1.00	1.02	4.94	1.20	1.11
	15.0	15.17	1.13	0.96	15.28	1.87	1.20
	25.0	24.76	0.96	1.08	25.31	1.24	1.15
Method C	4.0	3.94	1.50	1.14	3.91	2.25	1.21
	8.0	8.10	1.25	1.17	8.16	2.00	1.24
	12.0	12.13	1.08	1.00	11.73	2.25	1.09

RE. Relative error and RSD. Relative standard deviation.

The assay procedure was repeated seven times, and percentage relative standard deviation (% R.S.D) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. The placebo blank solution was subjected to analysis according to the recommended procedures. The resulting

absorbance readings for both the methods were same as the reagent blank, registering no interference from placebo.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution yielded percent recoveries which ranged of 97.58 - 101.1 with standard deviation of 0.62 – 1.26 in all the cases. The results of this study are presented in Table III indicating that the inactive ingredients did not interfere in the assay. These results further demonstrate the accuracy as well as the precision of the proposed methods.

Table III.

Recovery of the drug from synthetic mixture

Method	FMT in synthetic mixture taken $\mu\text{g/mL}$	FMT recovered ^a (Percent $\pm$ SD)
Method A	3.0	97.58 $\pm$ 1.26
	6.0	98.46 $\pm$ 0.72
	9.0	99.72 $\pm$ 1.04
Method B	5.0	100.3 $\pm$ 0.92
	15.0	101.1 $\pm$ 0.62
	25.0	100.8 $\pm$ 1.16
Method C	10.0	98.44 $\pm$ 0.76
	20.0	99.32 $\pm$ 0.82
	30.0	99.84 $\pm$ 1.05

^a Mean value of five determinations

Application to tablets

In order to evaluate the analytical applicability of the proposed methods to the quantification of FMT in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method [2] by applying Student's t-test for accuracy and F-test for precision. The reference method involved the titration of FMT in anhydrous acetic acid with acetous perchloric acid to potentiometric end point detection. The results (Table IV) show that the Student's t- and F-values at 95 % confidence level are less than the tabulated values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Table IV.
Results of analysis of tablets by the proposed method
Found^a (Percent of label claim $\pm$ SD)

Tablet Brand name	Label claim, mg/tablet	Reference method	Method A	Method B	Method C
Topcid-20 ^{®b}	20	96.58 $\pm$ 1.28	96.74 $\pm$ 0.85 $t=0.236$ $F=2.27$	97.32 $\pm$ 1.14 $t=0.97$ $F=1.26$	95.97 $\pm$ 1.28 $t=0.75$ $F=1.00$
Famocid-20 ^{®c}	20	98.36 $\pm$ 1.57	97.38 $\pm$ 0.92 $t=1.24$ $F=2.91$	99.53 $\pm$ 1.15 $t=1.36$ $F=1.86$	98.14 $\pm$ 1.03 $t=0.27$ $F=2.32$

^a Mean value of five determinations.

^b Torrent Pharmaceuticals Ltd., H. P, India; ^c Sun Pharmaceuticals Industries, Jammu, India.

The value of t (tabulated) at 95 % confidence level and for four degrees of freedom is 2.77.

The value of F (tabulated) at 95 % confidence level and for four degrees of freedom is 6.39.

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure FMT at three concentration levels (50, 100 and 150 % of that in tablet powder) and the total was found by the proposed methods. In all cases, the added FMT recovery percentage values ranged of 96.40 – 105.1 % with standard deviation of 0.78 – 1.75 (Table V) indicating that the recovery was good, and that the co formulated substance did not interfere in the determination.

Table V.
Accuracy assessment by recovery experiments

Method	Tablet studied	FMT in tablet $\mu\text{g/mL}$	Pure FMT added $\mu\text{g/mL}$	Total found $\mu\text{g/mL}$	Pure FMT recovered ^a Percent $\pm$ SD
Method A	Topcid-20 [®]	4.84	2.5	7.25	96.40 $\pm$ 1.75
		4.84	5.0	9.78	98.80 $\pm$ 1.28
		4.84	7.5	12.34	100.05 $\pm$ 0.84
Method B	Topcid-20 [®]	9.73	5.0	14.81	101.6 $\pm$ 1.28
		9.73	10.0	20.06	103.3 $\pm$ 0.94
		9.73	15.0	25.50	105.1 $\pm$ 0.78
Method C	Topcid-20 [®]	9.60	5.0	14.68	101.5 $\pm$ 1.26
		9.60	10.0	19.56	99.60 $\pm$ 0.85
		9.60	15.0	25.14	103.6 $\pm$ 1.52

^a Mean value of three measurements.

Conclusions

Three simple and fast UV spectrophotometric methods for the determination of FMT in pure form and in tablets were developed and validated as per the current ICH guidelines. Compared with most of the existing methods for FMT, the present methods are very simple, rapid and cost effective. The chromatographic methods [4-6], no doubt, are sensitive and selective; but require complex and expensive equipment, provision for use and disposal of large quantities of solvents, labour-intensive sample preparation procedure and personnel skilled in chromatographic techniques.

Of the non-chromatographic methods, the methods based on the potentiometry [8] and polarography [9] require strict pH control. Although the chemiluminescence method [10] is sensitive, it requires expensive experimental set up and the spectrofluorimetry method [11] is highly sensitive but requires strict pH control.

The reported visible spectrophotometric methods based on charge transfer and ion-pair complex formation reactions suffer from one or the other disadvantage like less sensitive, use of expensive reagent, use of organic solvents, strict pH control and involvement of liquid-liquid extraction step. The method based on condensation reaction [15] involves boiling step for 5 minutes and also the use of organic solvent. Redox reactions also require boiling step [16], narrow linear range [17] and poor sensitivity [18]. The bromination reaction [19] is very less sensitive and measured at shorter wavelength.

The UV-spectrophotometric method reported by R Sahu *et al.* [20] uses an organic solvent and is less sensitive (10-60 mg/mL). In contrast to the above published spectrophotometric methods, the proposed methods are very simple and sensitive with wide linear dynamic ranges. They can be used in quality control and routine analysis of FMT in pharmaceuticals since the methods are both precise and accurate, and free from common additives and excipients that might be found in commercial tablets.

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