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ORIGINAL PAPER

Spectrofluorimetric Determination of Famotidine in Pharmaceutical Preparations and Biological Fluids. Application to Stability Studies

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Abstract A simple, economic, selective, and stability indicating spectrofluorimetric method was developed for the determination of famotidine (FMT); is based on its reaction with 9, 10-phenanthraquinone in alkaline medium to give a highly fluorescent derivative measured at 560 nm after excitation at 283 nm. The fluorescence intensity - concentration plot was rectilinear over the concentration range of 50–600 ng/ml with minimum quantification limit (LOQ) of 13.0 ng/ml and minimum detection limit (LOD) of 4.3 ng/ml. The factors affecting the development of the fluorescence intensity of the reaction product were carefully studied and optimized. The method was applied for the determination of FMT in its dosage forms. The stability of the compound was studied, and the proposed method was found to be stability indicating one. The results obtained were in good agreement with those obtained by the official method. Furthermore, the method was applied for the determination of FMT in spiked and real human plasma. The mean % recovery (n=4) was found to be 99.94±0.24, and 105.13±0.64 for spiked and real human plasma, respectively. The composition of the reaction product as well as its stability constant was also investigated. Moreover, the method was utilized to investigate the kinetics of both alkaline and oxidative induced degradation of the drug. The apparent first order rate constant and half life time of the degradation product was calculated. A proposal of the reaction pathway was postulated.

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Introduction

Famotidine (FMT), 3-[2-(diaminomethyleneamino] thiazol-4-ylmethylthio]- *N*-sulphamoyl propionamidine (Fig. 1); is a histamine H_2 antagonist which is used in the management of benign gastric and duodenal ulceration, gastro-esophageal reflux, heart burn, and Zollinger–Ellison syndrome [1].

Literature survey reveals many methods for the determination of famotidine in pharmaceutical preparations and biological fluids including: spectrophotometry [2–9], polarography [10], HPLC [11–17], and fluorimetry [18, 19]. Although chromatographic methods offer a high degree of specificity, yet they require large amount of high purity organic solvents and generate high amount of waste. Therefore, there is a need for an alternative substitute to these techniques for the routine quality control analysis of famotidine.

The British Pharmacopoeia (BP) [20] recommended a potentiometric non aqueous method for the determination of FMT using perchloric acid as a titrant. While the United States Pharmacopoeia (USP) [21] recommended a similar approach for the determination of FMT in its bulk form, and an HPLC method for its determination in tablets using a mixture of acetate buffer: acetonitril (93:7) of pH 6 as a mobile phase with UV detection at 275 nm.

The molecular structure of FMT is characterized by the presence of guanide groups which may couple with 9,10-phenanthraquinone, and this initiated the present study. The proposed method is simple, rapid, and more sensitive compared with the reported spectrofluorimetric methods [18, 19]; it could be applied for the determination of FMT in pharmaceutical preparations, spiked and real human plasma.

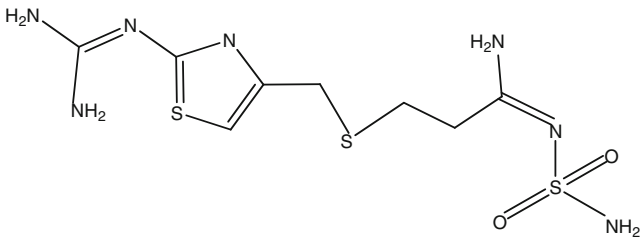


Fig. 1 Structural formula of famotidine

The reported spectrophotometric and spectrofluorimetric method for the analysis of Famotidine depends on its reaction with 1,4 Benzoquinone reagent at pH 5.2 [18]. The absorbance of the resulting condensation product was measured at 502 nm. The absorbance-concentration plots were rectilinear over the range 40–160 μg ml^{-1} Furthermore the resulting condensation products exhibited fluorescence at 665 nm after excitation at 290 nm and the calibration graph was rectilinear from 0.4–1.4 μg ml^{-1} On the other hand, the other reported spectrofluorimetric method for the determination of famotidine depends on the synchronous spectrofluorimetric assay of the drug in the presence of fluconazole and ketoconazole using methanol as a solvent [19]. The fluorescence intensity of FMT was recorded at 384 after excitation at 284 nm. The calibration curve was linear over the concentration range 15–50 μg/ml.

Experimental procedures

Apparatus

The fluorescence spectra and measurements were recorded using a Perkin Elmer LS 45 Luminescence Spectrometer equipped with a 150 W Xenon arc lamp. A 1 cm quartz cell was used.

Materials and reagents

All reagents and solvents were of Analytical Reagent grade.

1. Famotidine (FMT) was kindly provided by Memphis Pharmaceutical Company, Cairo, Egypt. Its purity was checked according to BP [20] and was found to be 98.8%.
2. Pharmaceutical preparations:
   - Antodine® ampoules (Batch # 22298) labeled to contain 20 mg famotidine/ampoule, and Antodine® tablets (Batch # 3728), labeled to contain 20 mg famotidine/tablet, produced by Amoun Pharmaceutical Company, El-Obour City, Cairo, Egypt.
   - Famotin® tablets (Batch # 384178), labeled to contain 40 mg famotidine/tablet, Memphis Pharmaceutical Company, Cairo, Egypt.
   - Servipep® tablets (Batch # 050), labeled to contain 40 mg famotidine/tablet, Novartis Pharma S.A.E, Cairo-C.C.R.111108 under license from Sandoz GmbH, Kundl-Austria.
   - Peptec® tablets (Batch # 044), labeled to contain 20 mg famotidine/tablet, manufactured by Julphar Agency, Egypt.
3. 9, 10-Phenanthraquinone (Fluka) was prepared as 0.1% (*w*/*v*) solution by dissolving 100 mg of the reagent in 100 ml of dimethylformamide (DMF) and further diluted with the same solvent to obtain a stock solution of 2 μg/ml.
4. Sodium hydroxide (BDH-Poole, UK), 1 M aqueous solution.
5. Hydrochloric acid (BDH-Poole, UK), 1 M aqueous solution.
6. Methanol (Aldrich) and dimethylformamide (BDH, Poole, UK).

Standard solutions

Stock solution was prepared by dissolving 100.0 mg of FMT in 100 ml of methanol and further diluted with the same solvent as appropriate. The standard solutions were stable for 7 days when kept in refrigerator.

General procedure

Aliquots of FMT standard solution covering the concentration range of 50–600 ng/ml were transferred into a series of 10 ml volumetric flasks. 0.5±0.05 ml of 1 M sodium hydroxide was added, followed by 2±0.4 ml of 9, 10-phenanthraquinone (2 μg/ml) and 0.5±0.05 ml of 1 M HCl, the volume was completed to the mark with distilled water and mixed well. The fluorescence intensity of the resulting solution was measured at 560 nm after excitation at 283 nm (Fig. 2). A blank experiment was prepared simultaneously for each measurement. The corrected relative fluorescence intensity was plotted *versus* the final concentration of the drug (ng/ml) to get the calibration graph; alternatively, the corresponding regression equation was derived.

Procedure for tablets

20 tablets were weighed and pulverized. A weighed quantity of the powdered tablets equivalent to 100.0 mg of famotidine was transferred into a small conical flask, extracted with methanol on three successive times each with 30 ml (3×30). The extract was filtered into 100 ml volumetric flask. The conical flask was washed with few milliliters of methanol and the washings were passed into the

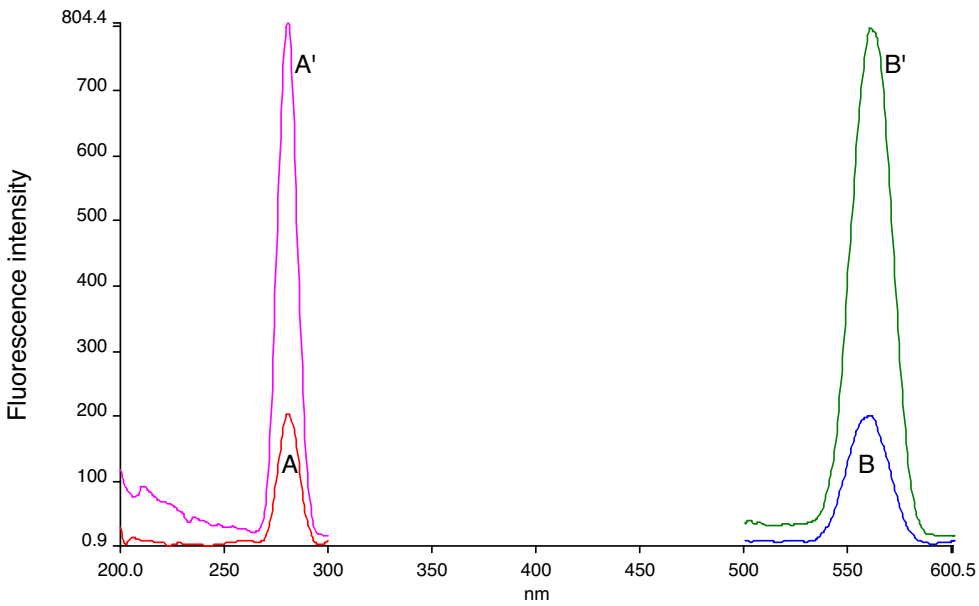


Fig. 2 Fluorescence spectra of the formed reaction product where: *A* and *B* are the excitation and emission spectra respectively of the blank reagent (2 μg/ml). *A'* and *B'* are the excitation and emission spectra respectively of FMT (400 ng/ml)–9,10-phenanthraquinone reaction product

same volumetric flask and the volume was completed with the same solvent. Aliquots covering the working concentration range were transferred into 10 ml volumetric flasks and the "General Procedure" was followed. The nominal content of the tablets was determined either from the calibration graph or by using the corresponding regression equation.

Procedure for ampoules

The contents of ten ampoules were mixed well, aliquot volumes equivalent to 100 mg of FMT were transferred into a 100 ml volumetric flask, serial dilution was performed with methanol to obtain the working concentration range and the steps described under "General Procedure" were followed. The nominal content of the ampoules was determined either from the calibration graph or by using the corresponding regression equation.

Procedure for preparation of degradation products

For the kinetic study, aliquot volumes of FMT standard solution were transferred into a series of 25 ml volumetric flasks to obtain a final concentration of 40 μg/ml, 0.5 M sodium hydroxide or 2% hydrogen peroxide were added. The solutions were left in a boiling water bath in case of alkaline degradation, and at ambient temperature in case of oxidative degradation for a fixed time interval (10 min). Aliquot volumes of the degraded solution were transferred to a series of 10 ml volumetric flasks and neutralized with 0.5 M hydrochloric acid for alkaline degradation, and the steps were completed as described under "General procedure". The relative fluorescence intensity of the resulting degradation products was recorded at 560 nm after excitation at 283 nm, log $a/a-\underline{x}$ *versus* time (minutes) was plotted to get the reaction rate constant and the half life time $t_{1/2}$. Complete degradation was attained by following the same procedure using 2 M sodium hydroxide or 30% hydrogen peroxide for alkaline or oxidative degradation respectively, followed by boiling for 1 h in case of alkaline degradation, and neutralization with 2 M hydrochloric acid. For oxidative degradation, the solution was allowed to stand for one hour at room temperature, and the excess hydrogen peroxide was removed *via* boiling in a water bath for 30 min.

Procedure for spiked human plasma

Aliquots, 1.0 ml, of human plasma were transferred into a series of centrifugation tubes, spiked with increasing quantities of FMT to give a final concentration range of 50–60 ng/ml. Then 3.0 ml of 1.2 mMol of trichloroacetic acid TCA (1×3) were added to precipitate plasma proteins, centrifugation at 3,000 rpm for 30 min was performed, the aqueous layer was transferred quantitatively to 10 ml volumetric flasks, and the steps described under "General Procedure" were performed.

Procedure for real human plasma

Servipep® tablet (40 mg famotidine/tablet) was administered to a healthy male volunteer (40 years old). After overnight fasting, 5 ml of the blood was withdrawn 3 h after administration of the drug, 5 ml of citrate were added, then centrifugation was performed to get about 2–3 ml of plasma. The steps described under "Procedure for spiked human plasma" were performed.

Results and discussion

9,10-Phenanthraquinone was utilized for the spectrofluorimetric determination of guanthidine in pharmaceutical preparations and biological fluids [22], and streptomycin in dosage forms and in spiked plasma [23].

Famotidine forms a highly fluorescent reaction product upon reaction with 9,10-phenanthraquinone in alkaline medium. The fluorophore was formed instantaneously and remained stable for more than 120 min.

Optimization of the reaction conditions

The spectrofluorimetric properties of the formed fluorophore as well as the different experimental parameters affecting development and stability of the reaction product were carefully studied and optimized. Such factors were changed individually while the others were kept constant. These factors include: concentration and volume of the reagent used, volume of NaOH, the effect of temperature, and different diluting solvents.

Effect of addition order

The effect of addition order on the fluorescence intensity of the system was studied. The results showed that the addition order of FMT-sodium hydroxide-9,10-phenanthraquinone–hydrochloric acid gave the best results.

Effect of different diluting solvents

Different diluting solvents were tested to choose the most suitable one for the formation of the reaction product, the investigated solvents included: water, acetonitrile, methanol, dioxane, dimethylsulfoxide, acetone, 0.1 M sodium hydroxide, and 0.1 M hydrochloric acid. The highest fluorescence intensity was achieved upon using water. Moreover, its choice adds another advantage to the method. The results are abridged in Table 1.

Table 1 Effect of different diluting solvents on the relative fluorescence intensity of the formed reaction product

Solvent	Relative fluorescence intensity (RFI)
Water	587
Methanol	363
Acetonitrile	529
Dimethylsulfoxide	415
Dimethylformamide	335
Acetone	20
0.1 M sodium hydroxide	450
0.1 M hydrochloric acid	430

Effect of concentration of 9,10-phenanthraquinone

The effect of 9,10-phenanthraquinone concentrations on the values of the relative fluorescence intensity of the reaction product was investigated, keeping all the variables constant. It was found that increasing the concentration of the reagent resulted in a gradual increase in the fluorescence intensity of the formed fluorophore up to (1.8 μg/ml) after which, it remained constant; therefore (2.0±0.2 μg/ml) was used throughout the study (Fig. 3).

Effect of volume of 9,10-phenanthraquinone

Keeping all the variables constant, it was found that increasing the volume of 9,10-phenanthraquinone (2.0 μg/ml), resulted in a gradual increase in the relative fluorescence intensity of the reaction product up to 1.6 ml, after which it remained constant, therefore, 2.0±0.4 ml of the reagent was chosen for the study (Fig. 4).

Effect of volume of 1 M sodium hydroxide

It was found that increasing the volume of sodium hydroxide (1 M) resulted in a gradual increase in the relative fluorescence of the reaction product up to (0.5–0.6) ml, after which it started to decrease gradually, therefore 0.5±0.05 ml of 1 M sodium hydroxide was used during this approach (Fig. 5).

Analytical performance and application

Using the above spectrofluorimetric method, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the fluorescence intensity on the concentration of the drug over the range cited in Table 2.

The relative fluorescence–concentration plot was found to be linear over the range of 50–600 ng/ml. linear regression analyses resulted in the following equation:

$$F = 1.46C + 0.30 \qquad (r = 0.9999)$$

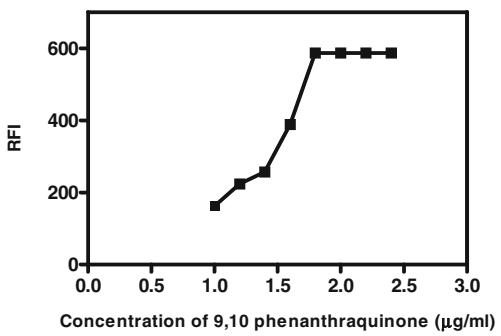


Fig. 3 Effect of concentration of 9,10-phenanthraquinone on the fluorescence intensity of FMT (400 ng/ml)–9,10-phenanthraquinone reaction product

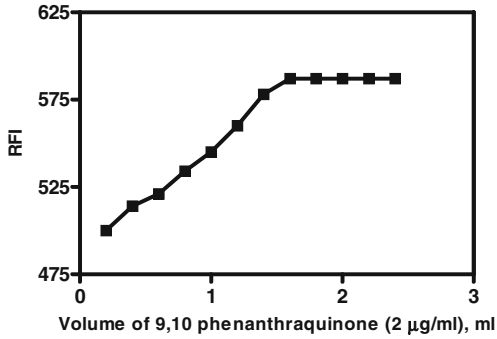


Fig. 4 Effect of volume of 9,10-phenanthraquinone (2 µg/ml) on the fluorescence intensity of FMT(400 ng/ml)–9,10-phenanthraquinone reaction product

Where F is the relative fluorescence intensity in 1-cm cell, C is the concentration of the drug in ng/ml, and r is the correlation coefficient.

LOQ and LOD were calculated according to ICH recommendations [24], and were found to be 13.07 and 4.31 ng/ml respectively.

LOQ was calculated according to the following equation [24]:

$$\mathrm{LOQ} = 10\sigma/\mathrm{S}$$

Where

σ the standard deviation of the intercept of the regression line.
S slope of the calibration curve.

The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected and it was calculated according to the following equation [24]:

$$\mathrm{LOD} = 3.3\sigma/\mathrm{S}$$

Validation of the method

The method was tested for linearity, specificity, accuracy and precision. Using the above spectrofluorimetric method,

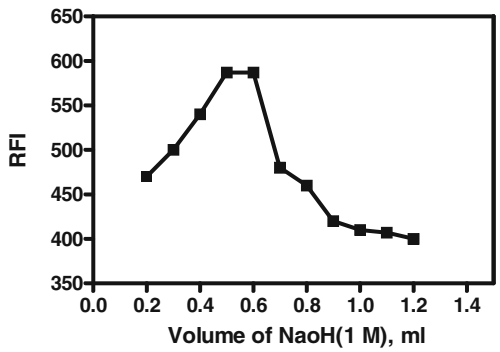


Fig. 5 Effect of volume of sodium hydroxide (1 M) on the fluorescence intensity of FMT(400 ng/ml)–9,10-phenanthraquinone reaction product

Table 2 Performance data of the proposed procedure

Parameter	Value
Concentration range (ng/ml)	50–600
LOD (ng/ml)	4.31
LOQ (ng/ml)	13.07
Correlation coefficient (r)	0.9999
Slope	1.464
Intercept	0.3011
Standard deviation of the residuals, $S_{y/x}$	3.08
Standard deviation of the intercept of the regression line, S_a	1.914
Standard deviation of the slope of the regression line, S_b	0.0056
% Error (RSD%/$\sqrt{n}$)	0.29
% RSD	0.67

linear regression equation was obtained. The regression plot showed that there was a linear dependence of the fluorescence intensity value on the concentration of the drug over the range cited in Table 2. The validity of the proposed method was evaluated by statistical analysis of the regression data regarding the standard deviation of the residual ($S_{y/x}$), the standard deviation of the intercept (S_a), and standard deviation of the slope (S_b) [25]. The results are shown in Table 2. The small values of the figures point to the low scattering of the points around the calibration graph and high precision of the proposed method.

Accuracy

The accuracy of the proposed method was evaluated by analyzing standard solutions of FMT. The results obtained by the proposed method were favorably compared with those obtained by the official method [21].

Statistical analysis [25] of the results obtained by the proposed and official methods using Student's t test and variance ratio F test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 3).

The proposed method was evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation (RSD %); the results are abridged in Table 3.

Precision

Repeatability

The repeatability was evaluated through analysis of 200 ng/ml FMT in pure or in dosage forms on four successive times. The mean percentage recoveries listed in Table 4 indicate the high precision of the proposed method.

Table 3 Application of the proposed and official methods to the determination of famotidine in pure form

Parameter	Amount taken, ng/ml	Amount found, ng/ml	% Found	Official method [21]
	100.0	100.51	100.51	100.23
	200.0	199.41	99.71	100.88
	300.0	301.32	100.44	99.65
	400.0	401.04	100.26	
	500.0	499.52	99.90	
$\bar{X}$ ±SD			100.16±0.35	100.25±0.62
Student's *t* test			0.41 (2.78)	
Variance ratio *F* test			3.09 (6.94)	

Each result is the average of three separate determinations.
Figures between parentheses are the tabulated *t* and *F* values respectively, at p=0.05 [25]

Table 4 Validation of the proposed procedure, for the determination of famotidine in pure and dosage forms

Sample	Percent recovery repeatability	Percent recovery (intermediate precision)
Famotidine (pure form)	100.23	99.78
	100.05	99.63
	99.98	100.61
	99.63	99.61
Mean found%	99.97	99.91
±SD	0.25	0.47
% RSD	0.25	0.47
Antodine ®ampoules (20 mg famotidine/ampoule)	100.32	100.34
	99.89	100.85
	99.64	99.61
	100.64	100.48
Mean found%	100.12	100.32
±SD	0.44	0.52
% RSD	0.44	0.52
Antodine ®tablets (20 mg famotidine/tablet)	100.63	100.64
	100.14	99.58
	99.63	99.67
	99.15	100.10
Mean found%	99.89	99.99
±SD	0.64	0.48
% RSD	0.64	0.48
Famotin®tablets (40 mg famotidine/tablet	99.63	100.54
	99.47	100.36
	99.64	100.84
	100.05	99.99
Mean found%	99.69	100.43
±SD	0.25	0.36
% RSD	0.25	0.36
Servipep®tablets (40 mg famotidine/tablet	100.21	99.63
	100.36	99.12
	99.63	100.05
	99.87	100.21
Mean found%	100.02	99.75
±SD	0.33	0.49
% RSD	0.33	0.49
Peptec® tablets (20 mg famotidine/tablet	100.32	100.11
	100.02	100.63
	100.22	100.78
	99.36	100.96
Mean found%	99.98	100.62
±SD	0.43	0.37
% RSD	0.43	0.37

Table 5 Application of the proposed and official methods for the determination of famotidine in its dosage forms

Pharmaceutical preparation	Amount taken (ng/ml)	Amount found (ng/ml)	Percent found	Official method % found [21]
Antodine®[a] ampoules (20 famotidine/amp.)	100	100.67	100.67	101.23
	200	199.95	99.98	100.35
	300	298.98	99.66	100.75
	400	400.25	100.04	
	500	500.59	100.12	
	600	599.67	99.95	
$\bar{X}$ ±SD			100.09±0.37	100.78±0.44
Student's t test			0.95 (2.31)	
Variance ratio *F* test			1.41 (5.79)	
Antodine®[b] tablets(20 mg famotidine/tablet)	100	99.95	99.95	99.65
	200	201.23	100.62	99.44
	300	300.85	100.28	99.12
	400	399.64	99.91	
	500	499.82	99.96	
	600	600.04	100.01	
$\bar{X}$ ±SD			100.14±0.30	99.40±0.27
Student's *t* test			0.37 (2.31)	
Variance ratio *F* test			1.23 (5.79)	
Famotin®[c] tablets (40 mg famotidine/tablet	100	99.12	99.12	100.36
	200	200.65	100.33	100.48
	300	300.29	100.09	99.48
	400	400.95	100.24	
	500	499.75	99.95	
	600	599.63	99.94	
$\bar{X}$ ±SD			99.95±0.48	100.11±0.55
Student's *t* test			0.83 (2.31)	
Variance ratio *F* test			1.31 (5.79)	
Servipep®[d] tablets (40 mg famotidine/tablet	100	99.63	99.63	100.55
	200	200.95	100.48	100.65
	300	299.36	99.79	99.65
	400	400.24	100.06	
	500	499.20	99.84	
	600	600.31	100.05	
$\bar{X}$ ±SD			99.96±0.33	100.28±0.55
Student's *t* test			0.86 (2.31)	
Variance ratio *F* test			2.78 (5.79)	
Peptec®[e] tablets (20 mg famotidine/tablet	100	99.12	99.12	100.45
	200	200.99	100.49	99.58
	300	300.24	100.08	99.65
	400	398.95	99.74	
	500	500.05	100.01	
	600	600.32	100.05	
$\bar{X}$ ±SD			99.89±0.51	99.89±0.48
Student's *t* test			0.02 (2.31)	
Variance ratio *F* test			1.12 (5.79)	

Table 6 Tolerance limits of co-administered drugs causing 3% relative error for a sample of famotidine 400 ng/ml

Drug	Fluorescence intensity	Tolerance limit (μg/ml)
Famotidine	587	–
Magaldrate	62	0.56
Dimethicone	40	0.36
Metformine	80	0.73

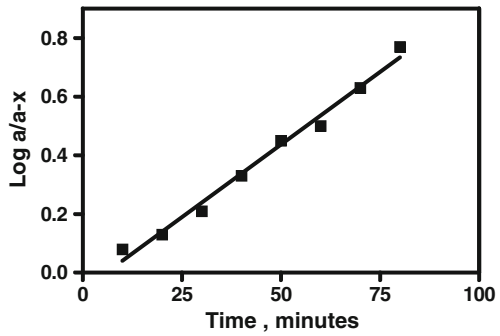


Fig. 6 Semi log plot of FMT (400 ng/ml)–9,10-phenanthraquinone reaction product versus different heating times (min) with 0.5 M sodium hydroxide at 100 °C

Intermediate precision

It was performed through repeated analysis of 200 ng/ml FMT either *per se* or in dosage forms on four successive days. The results are abridged in Table 4

Robustness of the method

The robustness of the method adopted was demonstrated by the consistency of the relative fluorescence values with the deliberately minor changes in the experimental parameters such as, concentration of 9,10-phenanthraquinone (2.0±0.2 μg/ml), volume of regent 2.0±0.4 ml of 2.0 μg/ml, volume of 1 M sodium hydroxide (0.5± 0.05), and volume of 1 M hydrochloric acid (0.5±0.05) did not greatly affect the fluorescence intensity of the reaction product.

Pharmaceutical applications

The method was successfully applied to the determination of FMT in its dosage forms.

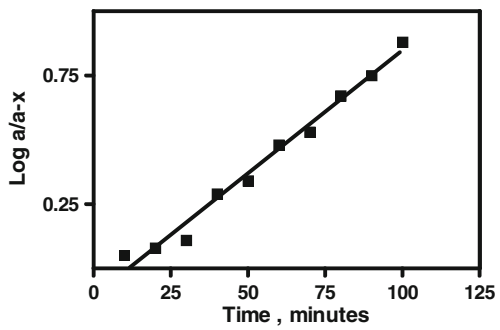


Fig. 7 Semi log plot of FMT (400 ng/ml)–9,10-phenanthraquinone reaction product versus different times (min) with 2% hydrogen peroxide on cold

Selectivity

Common tablet excipients such as talc, lactose, starch, gelatin and magnesium stearate did not interfere with the assay. The results are abridged in Table 5.

Accuracy

The results of the proposed methods were statistically compared with those obtained using the official method [21]. Statistical analysis of the results, using Student's *t* test and variance ratio *F* test, revealed no significant difference between the performance of the proposed and official methods [21] regarding the accuracy and precision respectively (Table 5).

Interferences

Many drugs which are frequently co-administered with FMT such as magaldrate, dimethicone, and metformine were carefully tested through using a solution of FMT and a solution of the studied compounds at a concentration of 400 ng/ml. The procedure mentioned above was applied for both FMT and the co- administered drugs. All of the studied compounds showed a negative interference as revealed by the low fluorescence intensity readings which proves that the studied method is specific for the determination of FMT only, excluding the studied drugs, under the specified experimental conditions. The results are abridged in Table 6.

The tolerance limits (concentration of interfering substance causing 3% relative error) of famotidine was calculated

The tolerance limit is an indication of the degree of interference of the co- administered drugs in the determination of FMT, the high values of the tolerance limits for magaldrate, dimethicone, and metformine shows that there is no significant interference in the determination of FMT by the studied method when these drugs are co- administered with FMT up to a concentration of 560, 360, and 730 ng/ml respectively; i.e., up to the calculated tolerance limit.

Formation constant of the reaction product was calculated according to the following equation:

$$K_f = \frac{F/F_m}{[(1-F/F_m)n+1]cn\,nn}$$

Where F and F_m: are the observed maximum fluorescence and the fluorescence obtained from the extrapolation of the two lines obtained from Job's continuous variation method, respectively.

n is the mole fraction of the reagent (the ratio is 1:2, as a result n=0.67)

Table 7 Application of the proposed method for the determination of famotidine in the presence of its oxidative and alkaline degradation products

Parameter	Amount taken of FMT (ng/ml)	Amount taken of degradation product (ng/ml)	Oxidative degradation product		Alkaline degradation product	
			Amount found of FMT (ng/ml)	Percent recovery	Amount found of FMT (ng/ml)	Percent recovery
	100	40	99.63	99.63	100.21	100.21
		60	99.10	99.10	99.67	99.67
		80	100.02	100.02	100.77	100.77
		100	99.76	99.76	99.24	99.24
		120	100.13	100.13	99.92	99.92
$\bar{X}$±SD				99.73±0.40	99.96±0.58	
Student's *t* test				0.30 (2.78)	0.37 (2.78)	
Variance ratio *F* test				2.40 (6.94)	1.14 (6.94)	
	300	100	300.92	100.31	300.32	100.11
		120	299.99	99.99	299.22	99.74
		140	300.46	100.15	300.64	100.21
		160	302.94	100.98	301.99	100.66
		180	299.11	99.70	299.78	99.93
$\bar{X}$±SD				100.23±0.48	100.13±0.35	
Student's *t* test				0.34 (2.78)	0.45 (2.78)	
Variance ratio *F* test				1.67 (6.94)	3.14 (6.94)	

Figures between parentheses are the tabulated *t* and *F* values, respectively, at $p=0.05$ [25]

C is the molar concentration of the drug used in Job's continuous variation method.

Using the above equation, K_f was found to be138×10^9

Also, Gibbs free energy changes (G) were calculated according to the following equation [26]:

$$\Delta G = -2.303RT \log K_f$$

Where

R gas constant = 8.3 J degree^{-1} mol^{-1}

T absolute temperature = °C+273

Using the above equation, ΔG was found to be -6.3×10^4 Kcal/Mole.

The negative value of ΔG indicates that the reaction is spontaneous.

Stability study

The proposed method is based mainly on the reaction between 9,10-phenanthraquinone and the guanido group of FMT. Therefore, degradation was attained upon induced alkaline degradation using sodium hydroxide (0.5 M) or oxidative degradation using 2% hydrogen peroxide. Upon

Table 8 Application of the proposed method for the determination of famotidine in spiked and real human plasma

Parameter	Taken (ng)	Found (ng)	Percent recovery
Intraday	50	49.91	99.82
Precision	55	55.12	100.22
(Spiked plasma)	60	59.87	99.78
$\bar{X}$±SD			99.94±0.24
Interday precision(spiked plasma)			
1st day	55	55.12	100.22
2nd day	55	55.23	100.42
3rd day	55	54.85	99.73
$\bar{X}$±SD			100.12±0.36
Intraday precision (real plasma)	156	165	105.77
	156	164	105.13
	156	163	104.49
$\bar{X}$±SD			105.13±0.64

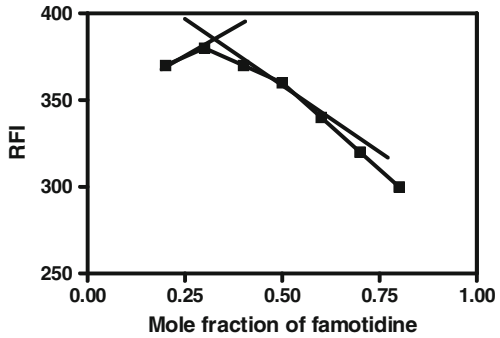


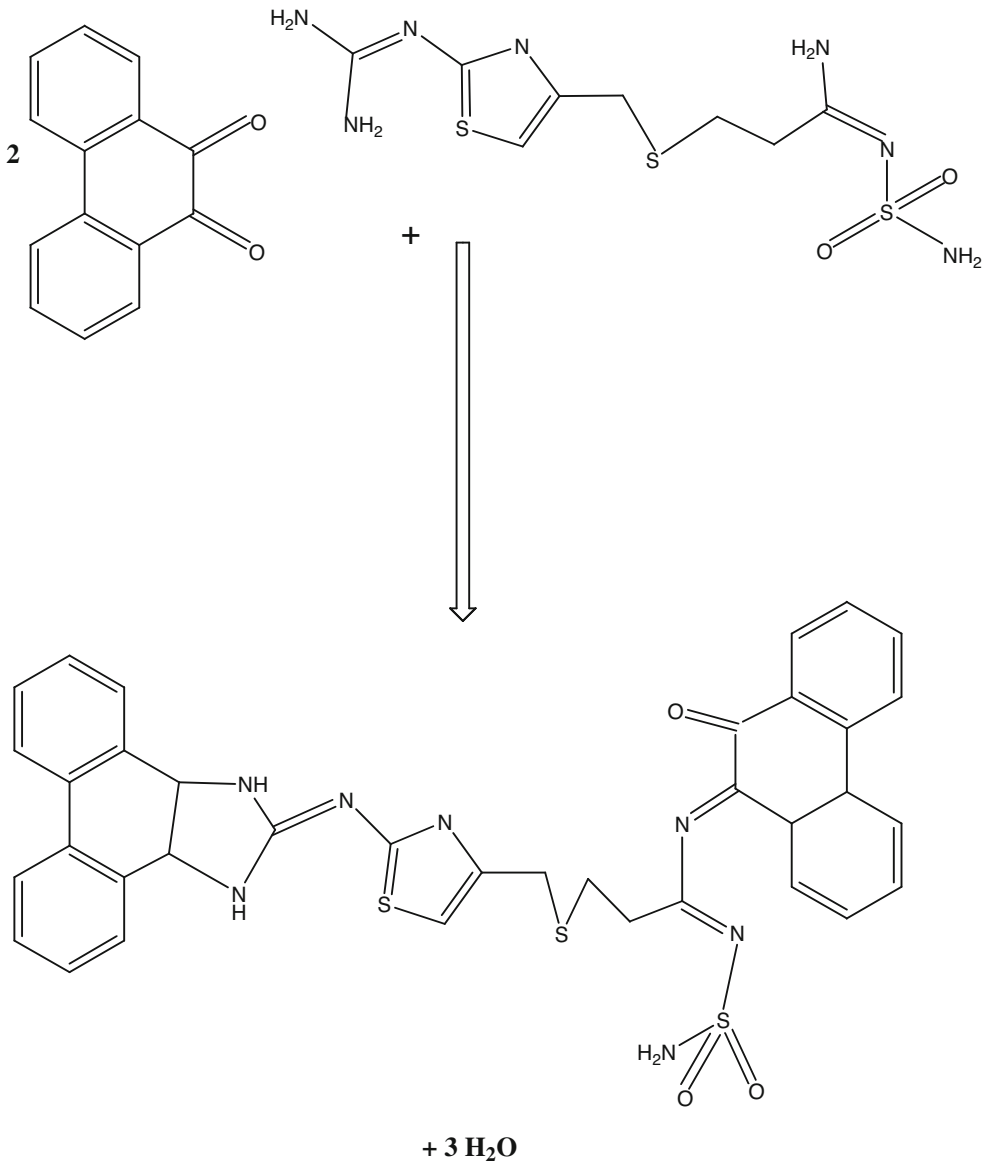
Fig. 8 Job's continuous variation method for FMT with 9,10-phenanthraquinone (9.6×10^{-5} M)

alkaline and oxidative degradation of FMT, the relative fluorescence readings of the fluorophore decreased gradually with time, thus indicating that the proposed method is a stability indicating one.

The induced alkaline degradation of FMT followed first order kinetics (Fig. 6) with a rate constant K=0.010 min^{-1}, and $t_{1/2}$=67.06 min. The oxidative degradation which also followed the first order kinetics had a reaction rate constant K=0.007 min^{-1}, and $t_{1/2}$=99 min (Fig. 7).

Complete alkaline or oxidative degradation was indicated from the disappearance of the fluorescence spectrum of the fluorophore. It was confirmed by performing a TLC scanning technique using a mobile phase consisting of chloroform: methanol: acetonitril (2:1:1) with UV detection, where the R_f values were found to be 0.81, 0.33, and 0.31 for famotidine, alkaline and oxidative degradation products respectively.

Scheme 1 Proposal of the reaction pathway between FMT and 9,10-phenanthraquinone



Complete alkaline degradation was attained after boiling with 2 M sodium hydroxide for one hour, while complete oxidative degradation was achieved through the addition of 30% hydrogen peroxide and allowing the reaction mixture to stand for one hour at room temperature. The results of statistical analysis of FMT intact drug in the presence of its degradation products are shown in Table 7. It is clear that the degradation products didn't interfere with the assay of the intact drug.

Upon exposure of methanolic solution of FMT to Deuterium lamp with a wavelength of 254 nm at a distance of 15 cm in a wooden cabinet for different time intervals (at a 10 min time interval up to 180 min), and then the studied method was applied, it was found that only 28% of the drug was degraded.

Application to spiked and real human plasma

FMT is readily but incompletely absorbed from the gastrointestinal tract with peak concentrations in plasma occurring 1 to 3 h after oral dose [1]. The serum therapeutic concentration range is 20–60 ng/ml [27], with a mean plasma level of about 156±22 ng/ml attained within 3 h [27]. These values lie within the working concentration range of the proposed spectrofluorimetric method, thus it could be successfully applied to the determination of FMT in both spiked and real human plasma over the working concentration range. The results are summarized in Table 8.

The within- day precision was evaluated through replicate analysis of FMT on three successive times, where the mean percentage recovery based on the average of 4 separate determinations was 99.94±0.24%.

The intra-day precision was evaluated through replicate analysis of plasma samples spiked with 55 ng/ml of the drug on three successive days. The mean percentage recovery based on the average of 4 separate determinations was 100.12±0.36%. The results are shown in Table 8.

Mechanism of the reaction

The stoichiometry of the reaction between FMT and 9,10-phenanthraquinone was also studied using Job's continuous variation method [28].As shown in (Fig. 8), the plot reached a maximum value at a mole fraction of 0.3 showing a 1:2 FMT-9,10-phenanthraquinone. The reaction pathway is proposed to proceed as shown in the Scheme 1.

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